AN ANALYSIS OF THE CHANGES INVOLVED IN A CASE OF PROGRESSIVE MUTATION[1]

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[Received June 14, 1917]

The relation of cell size to the size and organization of organisms has been investigated by many workers. The earliest and still one of the most important contributions to the subject was that of SANIO (1872), who studied the size of the cells in the pine. He found that at the base of the trunk the length of the tracheids increases in the first few annual rings to a maximum, which then remains constant. This length, which is constant at only one height, increases upward for a distance, reaches a maximum, and then decreases towards the top of the tree. The cells of the branches are smaller than those of the trunk but vary according to their position, following the same rule as those of the trunk.

It was SACHS who first appreciated the desirability of determining to what extent the size of an organ is correlated with the size of the cells composing it. In 1892 he set the following as the annual prize problem for the UNIVERSITY OF WÜRZBURG: "Es sind zahlreiche Messungen anzustellen, welche Auskunft darüber geben, ob und inwiefern Beziehungen zwischen dem Volumen der Zellen und dem Volumen der Pflanzenorgane bestehen." The prize was won by AMELUNG, who published his data in 1893. His chief conclusion was that in morphologically similar parts of plants the mean cell size remained so nearly constant that the difference in size of organs could be ascribed, without sensible error, wholly to the number of cells composing them. In this conclusion SACHS (1893) concurred, making the further deduction from AMELUNG's data that under normal conditions every plant or animal species attains a definite specific mean size, and that regardless of how much this specific mean size may vary from species to species, homologous cells are always of approximately the same size. In drawing these conclusions it is obvious that SACHS and AMELUNG disregarded fluctuating

[1] Papers from the Department of Botany of the UNIVERSITY OF MICHIGAN, No. 157.

variations as small as those which SANIO had found occurring within an individual.

STRASBURGER (1893) stated as a result of his work that just as the stronger or weaker development of an organ is without commensurate influence on the size of its constituent cells in their fully extended condition, so it is also without influence on the cells in their embryonic condition. He was struck by the fact that although individuals of the same species differed in number but not in size of cells, species of the same genus showed not inconsiderable deviations from one another. He added as an example *Lycopodium Selago, L. clavatum,* and *L. complanatum* and pointed out the large cell size of the Ranunculaceae, differing appreciably from other Dicotyledones and agreeing with the Monocotyledones.

As more critical work than that of SACHS and AMELUNG was done, it became obvious that these investigators had perhaps generalized more freely than their data warranted. GAUCHERY (1899) studied various cases of environmental nanism and found that even when other cell characters are unmodified, the diameter of the vessels is usually less in the dwarfs. The epidermal cells are ordinarily the same in dwarfs as in plants of the normal size (e.g., *Datura Stramonium*) but a dwarf of buckwheat proved an exception in having smaller epidermal cells. SCHNEGG (1902) published cell measurements of several species of Gunnera which are of interest chiefly because they so signally fail to substantiate the conclusions which their author draws from them. The genus Gunnera is remarkable for including species of ordinary size, together with others which are among the most gigantic of non-woody Dicotyledones. SCHNEGG extended AMELUNG's thesis to cover large and small species within the same genus, but his data, except with regard to the epidermis of the leaf, do not bear out his conclusion.

It would be fair to sum up the older work as follows: In general, robust plants differ from dwarf ones primarily in the number of cells of which they are composed, rather than in the size of the constituent cells, although environmental or internal factors that tend to accelerate growth also tend to increase slightly the size of the cells.

The most recent work has shown the necessity of taking nuclear characteristics into consideration in dealing with the relation of cell size to the organization of the individual. It is now known that accessions to the number of chromosomes in the nucleus bring about an increase in cell size, since the size of the cell may be considered as a function of the size of its nucleus. (See literature cited in the preceding paper

by TUPPER and BARTLETT (1918)). Consequently, in the cases where the difference between two species is primarily due to a difference in chromosome number, SACHS's conception is obviously inapplicable.

SIERP (1914) has made the most recent and exhaustive study of the relation of cell size to nanism, and has given especial attention to hereditary dwarfs. He makes a clear distinction between merely depauperate plants and true constitutional dwarfs, pointing out that his predecessor GAUCHERY had not, as he thought, dealt with the latter class of plants. He found that all depauperate plants had smaller cells than normal plants, but by no means smaller in proportion to the reduction of the plant size from the normal. The fact was established that the cells of a depauperate plant are not smaller than those of any juvenile plant of the same species, thus indicating that the difference is due to nutritional factors similar to those that are presumably concerned in the variation of wood cells in different parts of the pine. He compares his results with those of SANIO (1872), pointing out that the mean cell size increases from internode to internode, from a minimum at the base of the stem, where the cells are no larger than in depauperate plants, to a maximum at about the middle of the stem, and decreases to a minimum again at the apex of the stem. With regard to constitutional dwarfs, he found three conditions: (1) The cells are smaller than in the typical form (e.g., Solanum, Zea, Pisum, Clarkia). (2) The cells are of the same, or about the same, size (e.g., Mirabilis, Lathyrus). (3) The cells of the dwarf are larger (e.g., Nigella).

We may leave the third case out of consideration, as presumably one of cell gigantism due to chromosomatic modification (although in Primula there are cell giants that have the same number of chromosomes as the typical forms; may there not, however, be an increase of chromatin even in such cases?). The other two cases concern us more in the present paper. It has been possible to use the rich material presented by our Oenothera mutations to study cell conditions in constitutional dwarfs of known ancestry, grown under reasonably uniform conditions. It must be said in criticism of SIERP's admirable paper that he was unable to study dwarfs of known derivation, and that therefore he had no proof that the dwarfs with smaller cells than typical plants had actually sprung from strains presenting the same cell conditions as the non-dwarfs studied by him. In the two species whose dwarfs did not show a reduced cell size, the normal plants and dwarfs were undoubtedly of the same lineage. *Mirabilis Jalapa* is the only commonly cultivated species of the genus, and is relatively constant, aside from color varieties.

Lathyrus odoratus is likewise so well circumscribed that it crosses with no other species; it has given rise to its cultivated varieties, including the dwarfs, by mutation (BATESON 1913). In the cases of Zea, Solanum (group of *S. tuberosum*), Pisum, and Clarkia, the dwarfs are of unknown lineage, being derived from polymorphic groups within which there may well be considerable variation with regard to cell size. In the case of Zea, particularly, there is evidence (KUWADA 1911, 1915) that the chromosome number varies considerably from form to form, so that we should expect, *a priori,* a correlated variation in cell size. Aside from the occurrence of slight variations analogous to those of depauperate plants, there is little adequate evidence that hereditary dwarfs with the same chromosome number as the parent species ever have smaller cells.

In this paper we bring forward remarkably consistent evidence that hereditary nanism, in cases where the reduction in size is not associated with a changed chromosome number, is due to a diminished capacity for cell division. The cells of typical plants and dwarfs are potentially of equal size but differ in number and distribution. The series of forms investigated by us is remarkable in that the first three members show successive reduction stages from the typical individual of normal size to an extreme dwarf. We interpret these stages as exemplifying retrogressive evolution. The fourth member, however, shows a reversal of the direction of evolution. In the plant as a whole, the path of retrogression is partially retraced, and in one organ, the capsule, the original point of departure is passed, resulting in a net progression at the end of the evolutionary process. We are especially concerned in this paper with an analysis of the progressive mutation, as the title of the paper indicates, but the retrogressive mutations receive, incidentally, as complete a study.

Before presenting the data, our final conclusion may be stated briefly as follows: In a series of mutations showing both reduction and increase in the size of the entire plant and its organs, the size characters prove to involve only number and arrangement of cells, and not at all the size of the cells, which remains the same throughout the whole series.

In previous publications (BARTLETT 1915; LA RUE and BARTLETT 1917), we have described the origin by mutation from *Oenothera Reynoldsii* of three types of dwarfs, known as mut. *semialta,* mut. *debilis,* and mut. *bilonga.* They arise in the order in which they are listed, *Oenothera Reynoldsii* giving rise to mut. *semialta,* mut. *semialta* to mut. *debilis,* and mut. *debilis* in turn to mut. *bilonga.* The forms have also

all originated from *Oenothera Reynoldsii* directly. Mut. *semialta* shows considerable reduction from *Oenothera Reynoldsii* forma *typica* in both stature and leaf size, and may be characterized as a half-dwarf. Mut. *debilis* is a true dwarf with about a quarter the height of f. *typica*. It is not so greatly reduced as some of those derived from other species of Oenothera, notably *Oe. pratincola,* but is about as small a dwarf as any that are sufficiently fertile to be maintained in cultures. Mut. *bilonga* shows a progressive trend, returning to the stature of mut. *semialta*. It is therefore a half-dwarf. In the case of the capsules, there is a very remarkable progression, resulting in a capsule twice as long as that of the other half-dwarf, mut. *semialta,* and much longer than that of f. *typica* which forms the starting point of the evolutionary series.

Years ago SACHS (1893) made some interesting speculations in regard to the changes in the number of cells in an organism, the repetition of which may serve to indicate why we have interpreted increases in cell number as progressive evolution, and decreases as retrogressive. If one were to select from a genus of many species one typical of the genus, and of medium size, it would be possible to conceive of the changes that might follow either from increasing or decreasing the number of cells. In the former case the possibilities of variation would diminish as the number of cells diminished; simplification of structure and decrease of differentiation would inevitably follow if the process were carried far enough. In the latter case, however, it would be impossible to predict the differentiations and specializations that might result with increase in number of cells. The possibilities of evolution in the one case are limited, in the other case unlimited. We do not wish to be understood as denying that specialization may not sometimes accompany decrease in cell number, for it is well known that many small organisms are highly complicated and many larger ones are relatively simple. Nevertheless, we believe that the proposition is in general true, and that in the absence of other criteria, increase in cell number leads to greater complexity and may therefore be interpreted as evidence of progressive evolution.

Within the family Onagraceae we think no one would be inclined to doubt that Circaea is a more reduced type than Oenothera or Epilobium. It has presumably been evolved by reduction from the more generalized types like Oenothera. It has a meristic difference from other members of the family (two carpels instead of four). The same meristic variation occurs in *Oenothera pycnocarpa* Atkinson and Bartlett as a somatic mutation. Its most marked reduction, however, has been in the number

of cells comprising the capsule. The reduction has gone so far that the seeds are reduced to two in *Circaea Lutetiana,* and to one in the weakest and most reduced species of all, *Circaea alpina.* With the reduction of the fruit the mechanism for dehiscence has been lost, a simplification resulting as a natural sequence from reduction in the number of the cells. It is hard to see how further evolution can take place in the direction that has led to the genus Circaea. *C. alpina* is reduced almost to lowest terms, and unless a reversal of the direction of evolution occurs, the group must remain a terminal one.

The case of the genus Gaura is similar, although not so extreme. Here the meristic reduction has not occurred, the carpels remaining four in number as in the generalized types of the family. The reduction of the carpels has taken place, however, resulting in indehiscent fruits with only one or two seeds developing. In a tribe very closely allied to the Gaureae, and presumably descended from the latter, the meristic reduction to a 2-celled ovary has taken place. Moreover the same tribe, the truly remarkable Gongylocarpeae (J. DONNELL SMITH and ROSE 1913) shows us a new evolutionary trend, marked as we should have anticipated, by an increase in number of cells. The annual or biennial Gaura-like type has become perennial. The indehiscent fruits remain attached and become imbedded in the wood of the growing stem, giving rise, in the genus Burragea, to one of the most bizarre and unusual types in the plant kingdom. Here we find a good illustration of the truth of SACHS's remark that the possibilities of increase in cell number are hardly to be foretold.

We have therefore interpreted the change from dwarf to half-dwarf stature which occurs in the production of mut. *bilonga* from mut. *debilis* as progressive evolution. We view the increase in the length of the capsule of mut. *bilonga* as even more positive evidence of evolutionary progress. Authors have sometimes expressed the view that real progress would be indicated by Mendelian dominance of the more highly evolved type. There is no considerable body of positive evidence to support this view, which is based on the observation that recessives are very frequently weaklings, obviously defective, or unfitted to persist in the struggle for existence. Because of the existence of such a view, however, it is essential to point out the fact that the mutations forming the subject of this paper do not show Mendelian inheritance (LA RUE and BARTLETT 1917). If the view should turn out to be justified, it is interesting that mut. *bilonga* more nearly approximates the behavior of a dominant than any other Oenothera mutation except *Oe. rubricalyx*

Gates. Crossed with one another, the mutations and the parent form all give matroclinic progenies except when mut. *bilonga* is one of the forms involved. In that case, mut. *bilonga,* crossed with its immediate parent, mut. *debilis,* gives a matroclinic progeny when mut. *bilonga* enters the cross as the egg parent, and a dimorphic progeny consisting of both parent forms when it enters as the sperm parent.

We have no numerical data with regard to plant height of the mutations. Such data have, however, been kept for the dimensions of the leaves, which show exactly the same tendencies as the heights. Table 1 shows measurements of leaf length and leaf width in these forms. By comparing it with table 2, in which the capsule measurements of all the forms are given, it may be seen that the reduction in leaf size is nearly proportional to that of capsule length in mut. *semialta.* In mut. *debilis,* however, the reduction in capsule length is considerably less, proportionately, than that in leaf length and leaf width. The discrepancy between the percentage of reduction in stature and that in capsule size is still greater, especially in mut. *debilis.* These facts suggest that the factors determining fruit length may not be affected in the mutative process which gives rise to mut. *debilis* and mut. *semialta* but that the

TABLE 1

Length and width of leaves in Oenothera Reynoldsii and some of its mutations.

Length in mm	*typica*	*semi-alta*	*debilis*	*bilonga*	Width in mm	*typica*	*semi-alta*	*debilis*	*bilonga*
40-49	.	.	1	.	12-13	.	.	19	5
50-59	.	.	12	.	14-15	.	.	**75**	8
60-69	.	.	60	16	16-17	.	.	**72**	32
70-79	.	2	**101**	69	18-19	.	.	36	52
80-89	.	21	37	**110**	20-21	.	.	8	**79**
90-99	.	49	1	30	22-23	.	3	2	28
100-109	.	59	.	1	24-25	.	15		15
110-119	6	**73**			26-27	1	30		7
120-129	5	35			28-29	0	42		1
130-139	2	14			30-31	6	**51**		
140-149	19	3			32-33	4	**48**		
150-159	46				34-35	4	39		
160-169	**122**				36-37	5	21		
170-179	88				38-39	10	19		
180-189	14				40-41	34	3		
190-199	1				42-43	**66**			
200-209					44-45	**71**			
210-219					46-47	42			
220-229					48-49	17			
230-239					50-51	1			

TABLE 2

Length of capsule in Oenothera Reynoldsii and some of its mutations.

Length in mm	f. *typica*	mut. *semialta*	mut. *debilis*	mut. *bilonga*
14-15	.	.	7	
16-17	.	.	38	
18-19	.	.	86	
20-21	.	6	**105**	
22-23	.	43	**104**	
24-25	.	97	84	
26-27	.	**122**	51	
28-29	.	94	31	
30-31	4	57	12	
32-33	21	21	6	
34-35	65	13	3	
36-37	165	2	.	
38-39	**260**	1	.	
40-41	223	1	.	
42-43	82	.	.	1
44-45	10	.	.	0
46-47	.	.	.	4
48-49	.	.	.	8
50-51	.	.	.	49
52-53	.	.	.	87
54-55	.	.	.	122
56-57	.	.	.	130
58-59	.	.	.	**141**
60-61	.	.	.	110
62-63	.	.	.	71
64-65	.	.	.	36
66-67	.	.	.	18
68-69	.	.	.	5
70-71	.	.	.	3
72-73	.	.	.	1

slight reduction may be due to lessened vegetative vigor. This idea applies especially well to mut. *debilis*, which is a very weak form. When mut. *debilis* gives rise to mut. *bilonga* there is a doubling, not only of height and leaf size, but of capsule length as well. The resulting capsules, although borne by a half-dwarf, are the largest among the evening primroses, far exceeding in length those of any of the eastern American species with which experimenters are familiar. An inflorescence in fruit is shown at natural size in fig. 1.

It should be mentioned that table 1 is made up of measurements taken in 1916 from material grown in Michigan while those of table 2 were taken in 1915 from material grown in Maryland. This fact does not, however, affect the ratios between the four types since all capsules are compared with others of the same locality and season, and the same is true of the leaves.

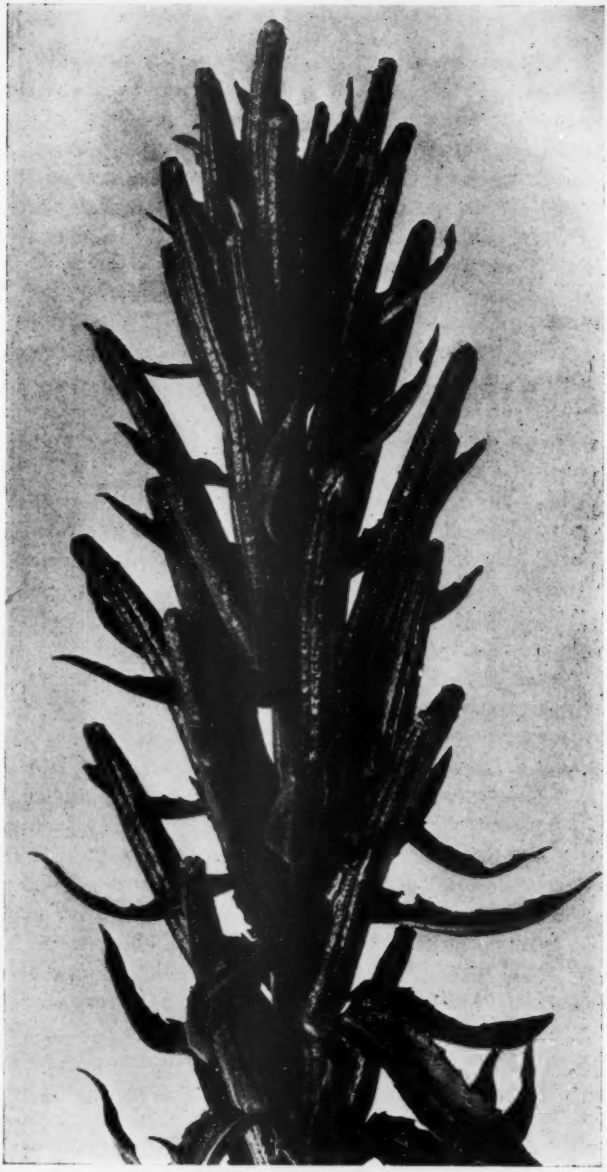

FIGURE 1.—*Oenothera Reynoldsii* mut. *bilonga*. Fruiting inflorescence, exactly natural size. The longest capsule is 64 mm long. The pedigree of this particular individual through six generations was: *typica*→ *typica*→ *typica*→ *debilis*→ *bilonga*→*bilonga*. (A member of culture 23; see LA RUE and BARTLETT 1917, p. 123.)

All of the forms show a considerable amount of sterility, as is common in Oenotheras. In muts. *semialta* and *debilis* the degree of sterility is greatest and these are the forms with the shortest capsules. In view of this fact a correlation of percentage of sterility and length of fruit seemed probable. Another explanation was possible, namely that capsule length might depend upon the total number of ovules rather than upon the number which develop. Mut. *bilonga* offered a more interesting case since the evidence given by it was expected to show whether or not the type represents a true progressive mutation. DAVIS (1915), in discussing these forms, suggested that a large increase in the total number of ovules in mut. *bilonga* over that in mut. *debilis* and the others would indicate a "true progressive advance," but should the increase in length be attended by an increase in the number of *fertile ovules* only it would indicate merely a reversion to an ancestral type having a higher degree of ovule fertility.

To determine the facts concerning the sterility of the ovules in these forms, counts of the sterile and of the fertile ovules were made in each of the four types. Capsules were selected for these counts of modal length as nearly as possible. All of these plants were growing in the same field and were of about the same maturity. The capsules were of mature size though the seeds were not fully ripened in any of them. The fruits were all gathered on the same day and were preserved in alcohol until the counts could be made.

The ovules were removed from the capsules, placed on a slide, and counted under a binocular microscope. After the ovules had been apparently removed from the capsules, it was found that many flat, aborted ovules still remained clinging to the placentae. It was therefore necessary to examine the placentae in all cases and count the ovules found there. This process was more troublesome than that of counting the other ovules and most of the errors made in counting were made here. Some errors were unavoidable as the interiors of the capsules are very mucilaginous and the very small, flat, aborted ovules cling together and to the larger fertile ones very tightly. The ovules were separated by fine needles and each was turned over to avoid overlooking any small attached ones. By using great care in this way it was possible to avoid any considerable percentage of error. In any case the errors should have been about the same for each of the forms and the large number of ovules in each capsule tends to make them less significant. Only those ovules were considered sterile which were entirely flat and obviously contained no embryos. Others, though small, were found to contain embryos and were classed as fertile.

Table 3 shows that mut. *semialta* and mut. *debilis* have about the same total number of ovules as forma *typica*. On the other hand the number of sterile ovules is greatly increased. The decrease in fruit length here must be due at least in part to an increase in ovule sterility. This increase in sterility may be due to decreased vegetative vigor and thus to decreased nutrition. This explanation seems more probable in the

TABLE 3

Number of ovules in the capsules of Oenothera Reynoldsii and its mutations.

Type	No. of capsule	Length in mm	Normal ovules	Sterile ovules	Total ovules
f. *typica*	1	39	404	383	787
	2	39	530	283	813
	3	38	446	389	835
	4	40	574	283	857
	5	39	537	208	745
	6	39	444	203	647
	7	38	516	289	805
	8	39	587	270	857
	9	39	537	293	830
	Mean	39	508	289	797
mut. *semialta*	1	26	116	620	736
	2	27	364	454	818
	3	28	163	496	659
	4	26	138	610	748
	5	27	195	593	788
	6	27	102	790	892
	7	27	253	484	737
	8	26	175	608	783
	9	26	141	508	649
	10	26	120	698	818
	Mean	27	177	586	763
mut. *debilis*	1	26	133	691	824
	2	22	17	505	522
	3	25	64	766	830
	4	18	158	319	477
	5	16	79	319	398
	Mean	21	90	520	610
mut. *bilonga*	1	52	669	591	1260
	2	55	688	484	1172
	3	53	604	571	1175
	4	52	660	293	953
	5	56	464	469	933
	6	58	524	726	1250
	7	54	821	526	1347
	8	53	830	405	1235
	9	58	742	418	1160
	10	53	654	439	1093
	Mean	54	666	492	1158

light of some recent work of DAVIS (1916) in which he tried the effect on fertility of cutting down the nutrition of plants of Oenotheras by removing leaves. He found that the reduction of nutrition had a very decided effect on the fertility of his plants.

Mut. *bilonga* is not normally so sterile as mut. *debilis* which produces it. Table 3 shows it to have about the same percentage of sterility as f. *typica*. The total number of ovules, on the contrary, is greatly in excess of that in f. *typica* so that the increase in length of fruit is correlated with an increased number of ovules rather than with an increase in fertility. It appears that the formation of this mutation has introduced a higher ovule number and that the type represents a real case of progressive mutation.

A study of the cells of the capsules seemed desirable for the purpose of determining whether or not the changes in capsule length were due to or accompanied by giant or dwarf conditions of the cells. KEEBLE (1912) described cell gigantism in Primula unaccompanied by an alteration in the chromosome number and the case of Nigella mentioned by SIERP (1914) may be similar. It seemed possible that such a condition of cell gigantism might account for, or at least accompany, the difference in capsule length between mut. *debilis* and mut. *bilonga*. Tables 4 and 5 show the results of measurements of spiral tracheids and fiber tracheids from capsules of each type. For this work modal capsules were selected, macerated, and the wood elements measured with an eyepiece micrometer. A mechanical stage was used to avoid duplication of measurements.

TABLE 4

Length of spiral tracheids from capsules of Oenothera Reynoldsii and some of its mutations.

Length in mm	f. *typica*	mut. *semialta*	mut. *debilis*	mut. *bilonga*
.0332-.0664	2	7	7	5
.0664-.0996	22	34	19	19
.0996-.1328	**43**	**55**	**37**	**45**
.1328-.1660	36	36	28	26
.1660-.1992	30	26	23	21
.1992-.2324	26	10	20	18
.2324-.2656	9	10	4	14
.2656-.2988	10	5	5	10
.2988-.3320	6	6	1	10
.3320-.3652	4	2	1	6
.3652-.3984	5	2	0	6
.3984-.4310	0	2	0	4
.4310-.4684	0	1	0	0

TABLE 5

Length of fiber tracheids from capsules of Oenothera Reynoldsii and some of its mutations.

Length in mm	f. *typica*	mut. *semialta*	mut. *debilis*	mut. *bilonga*
.1992-.2324	.	.	2	1
.2324-.2656	.	.	7	8
.2656-.2988	1	.	8	10
.2988-.3320	6	7	13	11
.3320-.3652	13	21	16	13
.3652-.3984	21	27	27	19
.3984-.4310	42	36	31	25
.4310-.4684	54	42	34	38
.4684-.4980	74	44	41	46
.4980-.5146	**78**	**50**	**60**	**73**
.5146-.5478	76	44	42	63
.5478-.5810	70	38	28	63
.5810-.6142	60	34	24	55
.6142-.6474	30	30	18	52
.6474-.6806	28	24	14	37
.6806-.7138	19	23	13	36
.7138-.7470	13	9	12	20
.7470-.7802	5	8	4	20
.7802-.8134	3	3	3	18
.8134-.8466	1	1	.	9
.8466-.8798	4	3	.	9
.8798-.9130	.	.	.	7
.9130-.9462	.	.	.	4
.9462-.9794	.	.	.	3
.9794-1.0126	.	.	.	2
1.0126-1.0458	.	.	.	0
1.0458-1.0790	.	.	.	2

The results given in tables 4 and 5 show that both types of tracheids have the same modal length in all four forms. The range of fluctuation is greatest in mut. *bilonga,* and the environmental effect of diminished nutrition is obvious in the case of mut. *debilis,* through the greater number of short cells; but on the whole the conclusion is obvious that the cells of all four forms are so similar in size as not to account in any degree for the differences of capsule length.

In order to trace the possible influence of cell size on plant height, mature normal stems of each type were selected and samples of wood were taken from the base of the stem. These samples were macerated and studied in the same way as the capsules but in this case only one type of cell was measured. Table 6 gives the result of this study. In no case was the wood of a type taken from the same plant as the capsule yet the results are the same. This is the more interesting because the wood of the capsule is primary, and that of the lower stem almost all secondary. From this evidence it appears that mutative pro-

TABLE 6

Length of fiber tracheids of wood at base of stems of Oenothera Reynoldsii and its mutations.

Length in mm	f. *typica*	mut. *semialta*	mut. *debilis*	mut. *bilonga*
.1328-.1660	.	.	.	1
.1660-.1992	.	1	.	0
.1992-.2324	.	2	.	1
.2324-.2656	.	2	5	0
.2656-.2988	1	4	8	2
.2988-.3320	3	5	12	8
.3320-.3652	7	9	19	15
.3652-.3984	7	27	33	25
.3984-.4310	16	33	56	30
.4310-.4684	31	43	65	40
.4684-.4980	32	47	66	44
.4980-.5146	**58**	**57**	**75**	**49**
.5146-.5478	40	27	49	13
.5478-.5810	36	21	27	11
.5810-.6142	23	9	14	4
.6142-.6474	13	1	7	0
.6474-.6806	6	1	5	1
.6806-.7138	5			
.7138-.7470	3			
.7470-.7802	3			

cesses which produce forms entirely distinct from the parent in general characters and genetic behavior may still not be sufficiently deep-seated to affect such conservative tissues as those of the xylem.

The object of using wood from the base of the stem was, of course, to eliminate errors from variation due to environmental factors within the plant. As already pointed out, SANIO (1872) and later authors have shown the existence of considerable place effects on cell size within the plant. Since SIERP (1914) has emphasized the juvenile characteristics, as to size, of cells at the base of the stem of large plants, and has shown that all the cells of dwarfs approximate the juvenile condition, one would naturally select the base of the stem, in studying a series of forms, as the region most comparable. In Oenothera, however, it is probable that place effects within the plant are, for wood tissues, negligible. The evening primroses are more or less well-marked biennials. While in the rosette state, before the stem is sent up, they become very strong and robust, and store an abundance of reserve food for the subsequent rapid growth. In *Oe. Reynoldsii* f. *typica* the rosette stage has a tendency to be transitory, but it is developed sufficiently so that it might be expected to produce a distinct modification of the juvenile character of the cells of the growing point. The dwarfs are almost pure annuals,

mut. *debilis* and mut. *bilonga* often failing to persist for even a short time in a rosette stage. The wood specimens studied were therefore not as strictly comparable as one might infer from their position in the stem. The identical modes of all the variation curves consequently afford all the more striking confirmation of our general conclusion that the retrogressive and progressive evolution in the mutation series of *Oe. Reynoldsii* is tied up with changes in the number and arrangement of cells, rather than with their size.

Samples of wood of such of the hybrids between the various forms as have been secured were also studied. The authors' studies on these hybrids are described in the paper cited elsewhere in this article. It was found that these forms, with one exception, show matroclinic inheritance. Since all the forms have the same type of wood cells, it was not to be expected that these would shed any light on the problem of matroclinic inheritance in the hybrids. Nevertheless it is highly desirable to know just what their behavior is. The studies of the hybrids were made in the same manner as those of the uncrossed types. It is noted in the paper on matroclinic inheritance that some of the possible crosses have not been secured. These are mut. *semialta* × mut. *debilis* and mut. *bilonga* × mut. *debilis*. In addition to these missing forms the hybrid mut. *debilis* × f. *typica* is lacking in the wood studies because all the plants of this form were very weak so that good specimens could not be obtained. Table 7 shows the results, which, in the main, are what might be expected from the data given on the parents. The one notable exception will be discussed later. It will be noticed that in the cross mut. *debilis* × mut. *bilonga* data are given on two forms. This cross gives twin hybrids, one of which resembles the ovule parent and the other the pollen parent. All of the crosses showed wood cells like those of the parents. An effect on the cells akin to heterosis (the vigor of heterozygosis) was the most that might have been expected if the crosses had been different from the parents. As we have shown (La Rue and Bartlett 1917) no such effect was discernible externally, and none could be discerned in the cells. This is just what might have been anticipated from the hypothesis of non-equivalent gametes, which we have elsewhere applied to the mutations in question.

The cross mut. *debilis* × mut. *semialta* departs from the others in its behavior and gives a bimodal curve. The first set of results aroused a suspicion of an accidental mixture of the material. So a second sample was macerated from the same plant. All possible care was taken to secure a thorough mixing of the cells but the result was the same. In

Table 7

Length of fiber tracheids of wood at base of stems of the mutation crosses of Oenothera Reynoldsii.

Length in mm	*typica* × *semialta*	*typica* × *debilis*	*typica* × *bilonga*	*semialta* × *typica*	*semialta* × *bilonga*	*bilonga* × *typica*	*bilonga* × *semialta*	*debilis* × *semialta* (lot 1)	*debilis* × *semialta* (lot 2)	*debilis* × *bilonga* (*debilis* twin)	*debilis* × *bilonga* (*bilonga* twin)
.1992-.2324									1		
.2324-.2656			1						1	2	
.2656-.2988			3	4	2				6	3	2
.2988-.3320		2	5	5	7		5	6	12	6	6
.3320-.3652		7	19	8	13	8	15	11	20	6	4
.3652-.3984	4	20	24	20	16	22	20	19	46	23	8
.3984-.4310	12	34	37	37	32	25	38	42	77	31	23
.4310-.4684	20	52	37	44	34	26	46	**55**	**97**	69	32
.4684-.4980	34	54	40	50	46	47	53	38	83	82	45
.4980-.5146	**60**	**67**	**60**	**66**	**69**	**61**	**58**	**40**	**89**	**93**	**73**
.5146-.5478	41	32	30	30	32	39	28	14	32	35	49
.5478-.5810	37	27	17	14	13	13	16	6	17	26	22
.5810-.6142	17	10	17	6	7	9	6	4	4	14	14
.6142-.6474	12	2	6	6	6	2	3	0	1	2	10
.6474-.6806	9	1	6	3	3	3	4	1		1	3
.6806-.7138		0	4	2	1	2		1			
.7138-.7470		1			2						

measurements involving a population of any sort a bimodal curve is interpreted as an indication of a mixture of types. In a single plant it may be explained in a similar way. If a mutation should occur in the form of a chimaera such a mixing would occur. In this case it would affect only a part of the plant, the remainder having the same curve of variability as the other types. Unfortunately the wood samples were taken before the seeds were ripe so that no seeds of this plant were secured. One can only speculate on the possible behavior of a mutation which affects only the internal structure. If there had been external evidence of its existence it would have been detected and saved, for the culture in which it occurred was repeatedly inspected by both the writers. It might have been possible to determine from the existing material whether the chimaera was of a sectorial or a mosaic character but, since the study must have ended there, it was not thought worth the trouble.

SUMMARY AND CONCLUSIONS

(1) The series of mutations derived from *Oenothera Reynoldsii* shows both retrogressive and progressive evolution with regard to plant height, retrogressive from f. *typica* through mut. *semialta* to mut. *debilis*, progressive from mut. *debilis* to mut. *bilonga*. The changes are all concerned with the number and arrangement of cells, not with the size of the individual cells.

(2) The length of the wood elements is the same in the various matroclinic mutation crosses as in the self-pollinated strains. The crosses do not show, either in external or internal characters, any vigor due to heterozygosis. No such effect would be anticipated in view of the considerations developed in a former paper, in regard to non-equivalent gametes.

(3) The mutation series shows a relatively small reduction in capsule length from f. *typica* through mut. *semialta* to mut. *debilis*. This reduction is interpreted as probably a physiological manifestation, the less vigorous forms having the capacity to mature fewer ovules into seeds, and therefore producing shorter mature capsules. The number of ovules is nearly the same in the three forms, but the sterility increases with the degree of dwarfness of the form. The wood elements of the capsules of all three forms are of the same size.

(4) The process which results in the formation of mut. *bilonga* involves an increase over any of the other forms in the number of ovules, but not an increase in fertility over f. *typica*. It is therefore interpreted as a case of progressive evolution. The increase in number of ovules

is accompanied by a doubling in the length of the capsule; and this doubling is due to an increase in number of cells rather than to an increase in the size of the cells. The capsule of mut. *bilonga* is the largest known among the small-flowered Oenotheras of the *biennis* alliance.

(5) Aside from the possibility that one bimodal curve may have indicated the occurrence of a sectorial chimaera with smaller cells, there is no evidence that the retrogressive and progressive evolutionary changes which have taken place in *Oe. Reynoldsii*, affecting plant height, capsule length, and number of ovules, are accompanied by significant alterations in such conservative tissues as the xylem.

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VARIATIONS IN THE NUMBER OF SOMATIC CHROMOSOMES IN *OENOTHERA SCINTILLANS* DE VRIES

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[Received July 13, 1917]

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INTRODUCTION

The attention of both plant and animal cytologists has been focussed chiefly upon the germ cells. Before the students of the somatic chromosome complexes lies the problem of determining whether the individuality of the chromosomes is maintained in the body cells, and if not, what changes take place and with what results. The questions of a cytological explanation of bud mutations and of the behavior of cells in regenerating tissues, are still almost untouched. And further, there lies before the

investigator of somatic chromosomes the analysis of the conditions of pathological mitoses, with the ramifications of the problem, which may indeed eventually give us a better understanding of the functions of the chromosomes than have the studies of normal division figures.

It is only when the investigator of somatic chromosomes has a sufficiently large number of properly preserved somatic figures to study and has subjected the chromosomes to every possible analysis that he is justified in taking up the cudgels for or against the theories that have developed around the many excellent studies of germinal complexes. There is an apparent and regrettable tendency of late on the part of some cytologists to attempt merely a correlation of their own findings with those of some classic work on another form, passing over, at times, variations that have been found, either because they were thought unimportant, because they were supposedly pathological, or perhaps, because they appeared impossible of interpretation. Since the physiological character of the chromosomes is as yet undetermined and since at present our only method of forming a conception of the activities of the chromatic material is through morphological studies in the broadest sense, it scarcely needs to be pointed out that uncritical work of the sort described is not likely to be productive of results.

In connection with certain investigations on the somatic chromosomes of plants and animals I began the study of the somatic complexes of the "mutants" of *Oenothera Lamarckiana* at the suggestion of Dr. BRADLEY M. DAVIS. It was soon found that *Oenothera scintillans* did not give constant chromosome counts in the somatic cells. A complete study was carried out on this form resulting in the observations recorded below.

In a problem of this character plants offer exceedingly favorable material as it is possible to follow selfed lines through a number of generations, to obtain mitoses at various growth stages and, finally, to study germ cells from the same individual from which the active somatic tissues were earlier taken. It is impossible to obtain such a complete chromosome history in any animal with which I am familiar.

Although there is a variation in the number of somatic chromosomes of *Oenothera scintillans* this fact does not undermine the theory of the individuality of chromosomes, as has been thought by some writers, who have found differences in the somatic numbers in other material. This matter will be considered later.

The cytological data published in the present article is of considerable interest in itself but I wish particularly to emphasize the fact that the

conclusions could not have been drawn without the methods that were developed for analysing the chromosome groups. These methods of analysis, I believe, have considerable possibilities for chromosome studies of the future in both plants and animals. By the use of them it is possible to rule out almost entirely the personal equation since wherever it may enter there is at least one or more mechanical or mathematical checks against it.

It is a pleasure to have the opportunity of thanking Dr. DAVIS for his kindness in preparing material from his garden at times when I could not be there, and for his constant interest, discussions and advice during the progress of the work. To Dr. MCCLUNG and to my fellow workers of the Zoölogical Laboratory of the UNIVERSITY OF PENNSYLVANIA I am indebted for the stimulus and help their interest and discussions have afforded me.

TECHNIQUE

a. Material

The small amount of work on the somatic mitoses (of animals at least), has probably been due to the difficulty that is so frequently experienced of finding dividing cells outside the reproductive organs. In animals, as I have pointed out (HANCE 1917 a, b), this difficulty is largely overcome when an individual is found that is in a "cycle of division" (the same holding true for both somatic and germinal tissues). When such material is obtained the worker is more than repaid for his patience, as division figures will be found almost without number. This "cycle of division" also occurs in the plants with which I have worked and time will be saved if specimens are discarded that do not show a fairly large number of dividing cells on the first slide examined.

Somatic division figures were found in the tissues of young ovaries, in the sepals, petals and root tips. The first three tissues were obtained from young buds on adult plants. The root tips were collected from plants in the rosette stage which were carefully numbered and followed to maturity to make certain of their identity. This precaution is necessary in the case of *Oenothera scintillans* since this plant breeds true in only a small proportion of its progeny.

b. Methods of preparation

The young ovaries and anthers were fixed in FLEMMING'S strong solution to which had been added about one percent of urea. Urea, in animal technique, has been found to increase the rapidity of penetration.

This material was collected for three seasons (1914, 1915 and 1916) from the same line of plants. The root tips were similarly prepared in the spring of 1916. The material was cut from seven to ten micra and stained in iron-alum haematoxylin.

The mitoses in the root tip are more easily studied than in the other tissues for the reason that the chromosomes are more clearly separated. It will be shown later that the chromosomes of the root tips are generally longer and more slender than those of the other body tissues, and probably better fixed because fluids may enter more easily and rapidly into the root. Differences in length and width of chromosomes are known to be produced readily in active tissues when the killing fluid does not enter rapidly. The methods of analysing the cytological data obtained from the study of *Oenothera scintillans* will be considered in connection with the results obtained by these methods.

As I shall discuss recent papers of Miss LUTZ (1916, 1917) at some length it is appropriate to consider the results of the fixation of her material. I am inclined to believe that the fixation of the chromosomes in Miss LUTZ's material was not as good as could be desired. Miss LUTZ writes of encountering cells, while hunting for the "diminutive chromosome," where "the chromosomes were too massed and tangled to determine whether or not the small body was present." I do not mean to imply that all of the active cells of her material were in this condition but the very fact that such cells were even considered would seem to indicate that this "tangled" state was rather more prevalent than it should be. In the present study I have found the chromosomes well separated. The massing of the chromosomes may be due to poor fixation, to improper treatment after fixation or to shrinkage in imbedding. It may seem trite to raise the old cry about fixation and artefacts, but after all, it is a very vital criticism and our cytological conclusions are worthless and must be discarded sooner or later when based on material in which the preserved structures do not very closely approximate those of living cells.

This brings up the question of the dimensions of chromosomes. Unless the varying widths of the chromosomes in the majority of the published papers on the cytology of the Oenotheras are due to imperfect technique, the investigators have been misled as to the true proportions by viewing each chromosome from but one focus. When this is done the chromosomes do certainly appear narrower at one point than at another and appear to taper at the ends. I am inclined to believe that if the various material were reexamined carefully it would be found that the

chromosomes have a constant width and that all the chromosomes of a cell are equal in diameter. I think that this is borne out by some of the drawings published in Miss LUTZ's (1916) papers, as in her figures 3 and 4. In these instances it seems possible that the chromosome plates may have been flatter (lying more nearly in one plane), the entire length of the chromosome being more nearly at one level and focus (compare my figure 5 with plates 2 to 7). In working on material in which the chromosome number is variable a cell that shows the slightest indication of having been cut should not be considered.

THE SOMATIC CHROMOSOMES

As has been stated above, the study of the somatic complexes of *Oenothera scintillans* soon revealed the fact that the number of chromosomes in the body tissues is not constant. Special care, of course, was taken to count chromosomes in uncut cells only. Counts have never been built up from portions of a cell lying in two or more sections as the chromosomes of Oenothera are easily displaced or dragged from the cell by the knife in cutting. This study has been based on the examination of 114 cells and these were perfect cells in which there could be no mistake in count through over-lapping or other sources of error. Consequently I feel certain of the results here published, more especially as I was able to apply from time to time several checks on the accuracy.

a. The chromosomes at metaphase

The number of somatic chromosomes ranges from fifteen to twenty-one. While this entire variation in chromosome number was not found within a single plant, the number in any one plant was *not* constant. In the root tips gathered in 1916 representative of the groups 15, 16, 17, 18, 19, and 20 were found in one plant. Since it was believed when the first lot of material was studied that these variations might be limited to the plants of that year, material was gathered from the same line of *scintillans* for two succeeding years to determine this point. The later plants exhibited the same cytological characteristics and presented cells showing the varying numbers of chromosomes in almost exactly the same proportions as did the plants of the first year. These statistics are graphically illustrated in figure 1. The cells possessing fifteen chromosomes are more numerous than any other type, being represented by 51 cells out of the 114 or 45 percent. Since there are so many more of the fifteen-chromosome cells and since they were not produced, as will be

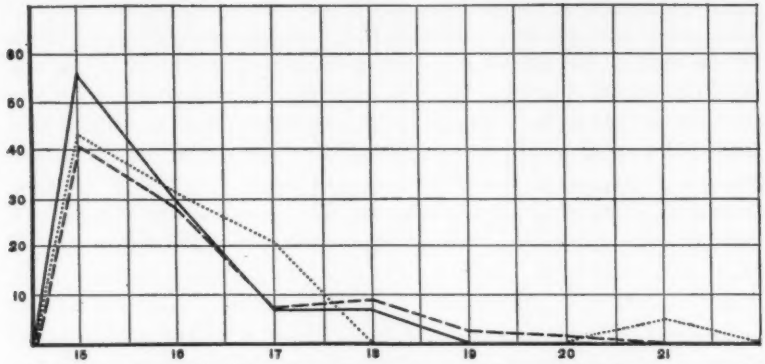

FIGURE 1.—A graphic illustration of percentages of the various classes (in respect to chromosome number) of somatic cells found in the material gathered in 1914, 1915 and 1916. The dotted line represents the 1914 data, the unbroken line the 1915 data and the broken line the 1916 data. The numbers along the base line refer to cell classes with respect to chromosome number and the numbers on the axis of ordinates indicate percentages. Note how nearly together are lines of the three years. These graphs are based on the following percentages: (the figures in parentheses refer to the numbers of cells studied).

Chromosome number	1914	1915	1916	Total number of cells
15	43% (8)	56% (15)	41% (28)	51
16	31% (6)	30% (8)	28% (19)	33
17	21% (4)	7% (2)	18% (12)	18
18		7% (2)	9% (6)	8
19			3% (2)	2
20			1% (1)	1
21	5% (1)			1
			Total	114

shown later, by a loss of chromatin, they are considered to be the *type group* and in this paper will be referred to as such. The types of the various complexes as represented on plates 2, 3 and 4 are:

15 chromosomes, Nos. 1, 2, 13, 16, 17
16 chromosomes, Nos. 3, 4
17 chromosomes, Nos. 5, 6, 15
18 chromosomes, Nos. 7, 8
19 chromosomes, Nos. 9, 10
20 chromosomes, No. 11
21 chromosomes, No. 12

What is the cause of this variation? At first it was thought that the extra number might be due to an early or precocious lengthwise division of a few of the chromosomes. Discovery of chromosomes that had begun to divide showed that this possibility could be ruled out, as the half chromosomes were noticeably narrower. Dividing chromosomes are shown in plate 3, No. 10, *b* and *c*. Observation determined that the metaphase plate could be accepted as a reliable stage in which to make the count. Was there some condition peculiar to somatic cells that brought about this irregularity of numbers? It was apparently something cytologically unusual for the somatic tissues since, as far as the Oenotheras studied are concerned, the condition is peculiar to *scintillans* except for the observations of Miss LUTZ (1916) which will be discussed later. Were any of the chromosomes passing to one pole undivided, or in other words, was somatic non-disjunction taking place? No evidence of this was found. Finally, were the chromosomes breaking up into shorter lengths—fragmenting? Instances of apparent fragmentation were soon found. Evidence of this process was shown in the constrictions that appeared in some of the chromosomes. These constrictions are very definite narrow areas in the chromosomes which run through its body and are not optical illusions gained from a single focus. They are illustrated in plate 2, fig. 1, a, fig. 3, a and b, and in plate 3, fig. 10, a, and it is interesting to note that figure 3, a, shows a chromosome with more than one constriction, the only case so far observed. Figures 9 a and 11 a, b and c, plate 3, are cases where the constriction has apparently broken through and the fragments retain their relationship and alignment. It frequently happens that the relationship of the chromosome fragments to one another is soon lost by their separation. Figure 4, plate 2, is a drawing of a much decolorized cell which shows the chromosomes a, b, c and d to be made up of a number of dark chromatin lumps between which are light areas. This is, of course, nothing unusual, but the lighter areas may be the points at which the constrictions in *scintillans* occur and at which points the chromosomes fragment.

A preliminary study of the root tips of a fourth generation of *Oe. scintillans* leads me to believe that here the increase in chromosome number is not as great as just indicated. Breaks or clear areas occur in certain chromosomes which make what may be a single chromosome appear as two. In my earlier material the possible relationship of chromosome portions on either side of the clear space was not as clear as in the latest specimens. The root tips collected present these portions in such

perfect alignment as to suggest that they are a part of a common body. The clear areas are variable in extent and in certain cases one portion of a chromosome may be turned at right angles to the other. In a few instances the separation seems to be complete. As I have not yet determined the nature of these clear intervals in the chromosomes all the separate chromatic bodies are considered in this paper, for the sake of analysis, as individual chromosomes or parts of chromosomes.

I have failed to find clear parallelism of the individuals of chromosome pairs such as GATES (1915) believes to be present in Oenothera. It is true that there are occasional chromosomes of about equal length lying side by side but I have not been able to find evidence of general or consistent arrangement in pairs. When the parallel arrangement of chromosomes is discussed it should only be of cases comparable to the striking types found in the Diptera (fruit flies and mosquito) (HANCE 1917, WHITING 1917) or something equally clear. This certainly does not occur in *Oenothera scintillans,* as is evidenced by the figures on the plates.

b. The chromosomes at anaphase

It was hoped that the study of the chromosomes at anaphase would throw some light on the behavior of the supernumerary chromosomes. Fortunately a number of excellent polar views of anaphases were found in the root tips, the chromosomes of which could be readily counted. Occasionally the anaphase groups had been cut and lay in two sections but these were only accepted as perfect when no evidence of disturbance could be seen. The majority of the cells in this stage were uncut. The anaphase groups lie in very flat plates and it is easy to focus from one group to the group beneath. All the anaphases counted showed either fifteen or sixteen chromosomes in each plate. It was disappointing not to have found some of the cells dividing, which possessed more than 16 chromosomes, but since cells with fifteen and sixteen chromosomes make up about eighty percent of all the various types of cells, it is obvious that anaphase figures with larger numbers of chromosomes in positions favorable for drawing and counting must be infrequent. However, the fact that the sixteen-chromosome class (which possesses *one extra chromosome*) is found with all of its chromosomes dividing regularly indicates that the one fragment behaves as a chromosome and divides as do the normal bodies. I think that we are justified in assuming that in the other classes with 17, 18, 19, 20 and 21 chromosomes the fragments behave similarly.

THE MEASUREMENT OF THE CHROMOSOMES

With the fact firmly established that some of the cells of *Oenothera scintillans* possess additional chromosomes and that these extra chromosomes are apparently the result of fragmentation of some of the fundamental chromosomes it became of interest to determine the following facts:

1. What is the relation of total length of all the chromosomes in those cells possessing the higher numbers to the total length of the chromosomes of the type group?

2. Do the chromosomes on fragmenting undergo any change? Have they the power of independent growth or do they break down and disappear?

It is evident that if it can be shown that the total length of the chromosomes is the same in the cells of all classes, the observational data (constrictions, etc.) which presented some evidence of fragmentation would be strikingly substantiated. Should this prove to be the case the second question would receive its answer in the same demonstration, as it has already been shown that the extra chromosomes divide regularly and are to be found in the anaphase of mitosis.

a. Methods of measuring the chromosomes

The fact that many of the Oenothera chromosomes are curved made it desirable to measure the drawing rather than the actual chromosome under the microscope. The chromosomes were drawn as carefully as possible with the aid of the camera lucida, at a magnification of 2800 diameters. Although they are absolutely distinct at this magnification the chromosomes are small and it was believed that greater accuracy would be obtained in measuring if the drawings were greatly enlarged. The cells in metaphase which showed the best separation of chromosomes and the flattest plates (this being necessary to obtain the full length of the chromosomes) were selected and the drawing was carefully enlarged six times with a pantograph. This gave a total magnification of about 17,000 diameters. Each chromosome was drawn separately so that the figures might be cut apart and arranged in a series according to length.

Curved chromosomes may be measured either with a string or by means of a notched wheel the teeth of which are definitely spaced. When these teeth are inked and rolled down the center of the chromosome a dotted line remains and the length of the chromosome can be determined by counting the dots and multiplying by the distance between them.

Either method is slow and the string method may be inaccurate if not employed with care. When hundreds of chromosomes are to be measured a method is desired which will be not only accurate but rapid. The automatic map-measuring device called the rotameter makes an admirable tool for this work. I have tested the one I have used against lines of known length and have found the results exceedingly accurate. All that is required when using the rotameter is to set the indicator at zero, roll the small wheel along the middle of the chromosome and read on the dial the length that has been traversed. It is as easy to measure the most complicated curve as it is a straight line. The method gives quick and reliable results. To further satisfy myself as to the accuracy of the device all chromosomes that were sufficiently straight were measured with the rotameter and the results were checked up with a scale.

b. The total lengths of the chromosomes

The chromosomes after being measured were inked in and arranged according to length on plates 6 and 7, so that they might better be studied and compared. Arranged in this way the pairing is quite marked in the type cells and there is a gradual but definite diminishing in the length of the chromosome pairs. This is a condition that is not at all obvious in the grouping at metaphase and, in fact, I had not realized in my previous studies on Oenotheras that there was any marked difference in the length of the chromosomes. The difference is slight and easily escapes notice when the chromosomes are not arranged for comparison as was done in this case. This fact very easily explains why the chromosomes of the Oenotheras have, so far as I am aware, generally been considered to be equivalent in length. This is borne out by the following statement of Miss Lutz (1916) on chromosome individuality.

"Gates found that the chromosomes of Oenothera present no constant differences in size and shape, and, with the exception of the small chromosome in the 14^{II}-chromosome mutants, the observations of the writer are in full accord with those of Gates."

When the lengths of the chromosomes of each class are averaged and added, the total lengths for the various classes proved to be almost identical. There is a slight variation but this is, I believe, well within the limits of technical errors. The results of these computations are given in figure 2. In this chart the average total length for each class has been drawn as a solid black column and it is obvious that the columns are very nearly of an even height. The "longest" example found in each group

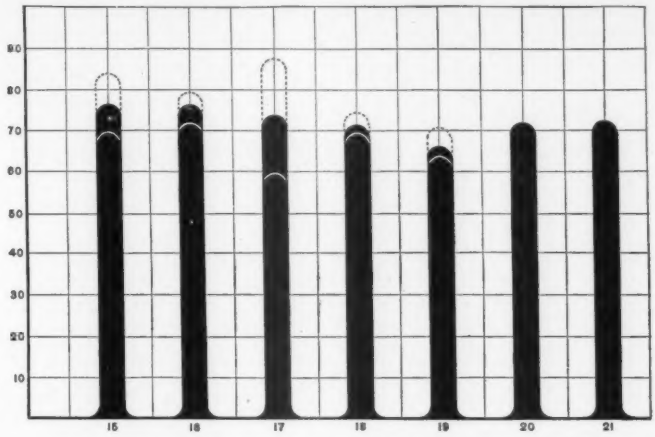

FIGURE 2. A graphic comparison of the total chromosome lengths in the various classes of cells. The black column represents the average lengths, the dotted lines above these columns represent the "longest" cell found in a group and the white lines across the pillars indicate the "shortest" cells. The charts are based on the data in tables 13 and 14, plates 6 and 7. Note how nearly of even height are the various columns.

is indicated by the dotted lines extending above the solid black pillar and the length of the "shortest" chromosome found in the group is indicated by the white line across the black column. The "19" column falls below the others, but it is based on an average of measurements of only three cells in which the chromosome plate was not as flat as would be desirable and where there was evidently some foreshortening. If the cell of the 19-chromosome group possessing the longest chromosomes (plate 7, row 20), were selected, the results would be indicated by the dotted line in figure 2. This would place the length of the "19" column very near the average length of the others. The variation in total lengths is so small that it seems justifiable at present to believe the difference to be entirely due to foreshortening.

Since the totals of the chromosome lengths appear to be approximately the same in all cases we must conclude that the increase in the number of chromosomes must be due to fragmentation. Furthermore these facts would indicate that the fragments retain their size and individuality in that they neither grow larger nor tend to degenerate and disappear. Additional confirmation of the last statement is to be found in the observations of the anaphase chromosomes of the "16" class, which show

clearly that the fragments here have divided in a regular way.

It will be seen in the tables accompanying plates 6 and 7 that the chromosomes P to U are all short, and when the length of these chromosomes (fragments) for each group is averaged, the results are fairly close.

Average length in centimeters of the chromosome fragments P to U

Group 16	2.7
Group 17	2.7
Group 18	2.4
Group 19	1.8
Group 20	2.0
Group 21	2.1
Average	2.3

Although convincing evidence can not be offered at present the results of my metrical studies have led me to believe that a chromosome generally does not lose more than one piece or section.

At this point it may be pointed out that the term "fragments" is applied only to those chromosomes in columns P to U. It is, of course, perfectly obvious that the remainders of chromosomes from which these fragments were separated, are also fragments. For convenience, however, the term is applied only to the chromosomes designated.

c. The somatic chromosome pairs

The fact that somatic chromosomes exist in pairs, which is not at all obvious when the chromosomes are grouped in the cell, is brought out rather markedly when they are arranged in a series according to length. This condition may be better realized by referring to the table of lengths accompanying plates 6 and 7. Individuals of a pair may vary to a greater or lesser extent but I believe that foreshortening may account for this. When an average of a number of cells is made it can be seen that the individuals of a pair are very nearly of even length. The pairing, as would be expected, is much more marked between the chromosomes of the type cells than between the chromosomes of cells, the seriation of whose chromosomes has been greatly disturbed by fragmentation. The pairing in these cells will be considered later.

In *Oenothera scintillans* there are seven pairs which will be designated as AB, CD, EF, GH, IJ, KL, and MN, and the unpaired chromosome O. Between these pairs there is a variation of length of approximately nine percent which appears fairly constant. A smaller pair of chromosomes is about 91 percent of the length of the next longer pair which in turn varies

from the pair longer than it by about the same amount. This relation will be considered later in connection with the analysis of the chromosomes.

THE ANALYSIS OF THE CHROMOSOMES

It is evident that the chromosomes of cells possessing more than the type number, do not as they are placed on plates 6 and 7, necessarily fall under the letter to which they properly belong, since they have there been arranged merely with reference to length. As fragmentation has occurred the natural order or sequence has been considerably disturbed. The questions which then took form were *whether it could be determined which chromosomes had fragmented and whether the chromosomes involved were the same in all cases.* If this could be discovered it might be possible to reunite the fragments so as to produce an approximation of the normal lengths and relationships found in the type group. Two methods have been employed in the attempt to determine which chromosomes had fragmented. They will first be described and the discussion of their respective merits will follow.

The chromosomes P to U on plates 6 and 7 all fall below the average length of chromosome O, the smallest chromosome of the type group, and consequently, as a working hypothesis, may be considered as the fragments of the larger chromosomes.

a. The first method of analysis

To determine whether the first fifteen chromosomes of each row as arranged on plates 6 and 7 were in their proper order or columns the lengths of the chromosomes A to O in the cells possessing extra chromosomes were compared with the *minimum length* of the chromosomes of the type group which fell under the same letter. The minimum length of the type group chromosomes was used for comparison as there seemed a greater possibility of this method giving accurate results than would a comparison with either the maximum or even the average lengths. This was believed to be true since a chromosome, even though in its proper place in the series, might well be shorter than even the average length for that column. Consequently if the comparison was with the average or maximum lengths chromosomes properly located and complete might frequently be judged to be fragmented. Comparing the chromosomes then to the minimum lengths those that fell below it, on it, or very close to it, were checked. Then the chromosomes which fell furthest below the minimum or, in case none were below this standard, those on or closest to it were selected for uniting with the fragments. These were considered

as chromosomes that had lost a portion of their length by the separation of the fragments. To those which differed most from the type minimum the longest fragment was added and so on down the list until all the fragments had been utilized. In reassembling these fragments the only criterion or check on the union of any two fragments was that the total length of the joined fragments should not exceed the *maximum length* found in the type group, which was seven centimeters for the enlarged figures. After the reassembling had been accomplished in any cell possessing extra chromosomes, all the chromosomes of that cell were arranged according to the new lengths obtained through the joining of the fragments. When this was done it was found that the new grouping closely approximated the average of the type group in many cases. This is illustrated in plate 1, figures 3 to 9, where the reconstructions and rearrangements have been made. It may be readily seen that the curves formed by the tops of the chromosomes in the rearranged groups is remarkably like that of the type group. Furthermore the new arrangement gave very nearly the same average variation between pairs as was found for the average of the type group. In table 1 the numbers opposite the chromosome pairs refer to the length in centimeters and the numbers between any two pairs give, in percent, the length of the shorter in comparison with the next longer pair.

TABLE 1

Pairs	Total number of chromosomes						Average percent
	16	17	18	19	20	21	
AB	6.5	6.9	6.4	6.7	6.8	6.6	
	89%	84%	92%	89%	92%	92%	89%
CD	5.8	5.8	5.9	6	6.3	6.1	
	95%	93%	88%	85%	87%	100%	93%
EF	5.5	5.4	5.2	5.1	5.5	6.1	
	91%	92%	96%	78%	89%	87%	88%
GH	5	5	5	4	4.9	5.3	
	96%	92%	94%	95%	84%	74%	89%
IJ	4.8	4.6	4.7	3.8	4.1	3.9	
	98%	93%	85%	84%	95%	95%	91%
KL	4.7	4.3	4	3.3	3.9	3.7	
	88%	91%	82%	88%	85%	84%	86%
MN	4.2	3.9	3.3	2.9	3.3	3.1	
	88%	84%	85%	86%	76%	97%	86%
O	3.7	3.3	2.8	2.5	2.5	3	
Average percent	92%	89%	89%	86%	87%	90%	

As can be seen in the table, individual pairs differ widely in certain cases, although in others they are fairly close to the type variation. The average variation between pairs differs somewhat from that of the type group.

b. The second method of analysis

The average length of the chromosome fragments, P to U, is 2.3 centimeters. When this length is subtracted from even the longest chromosomes that have been found in *scintillans* the remaining length is about equal to the length of the chromosomes at the short end of the series. In most cases, especially if some of the shorter chromosomes have lost a portion, the new lengths would be shorter than the smallest chromosomes of the series. In cells in which some of the chromosomes have undergone fragmentation, the chromosomes that are normally at the foot of the series, would, in practically all cases, be moved forward taking the place of chromosomes that had lost their position in the series through a reduction of their lengths. Consequently it seems justifiable to assume that in such an arrangement the main body of the chromosome, from which a portion had been separated, would lie somewhere near the short end of the series, if not actually at this end.

To test this hypothesis the chromosomes P to U were added in order to the chromosomes at the short end of the series. As will be seen by referring to the tables of lengths there is never any danger of exceeding the type maximum length in these additions. For example, if three chromosomes had fragmented, the fragments would lie in columns P, Q and R. These would be added to the chromosomes at the end of the series—P to M, Q to N and R to O. There is, of course, no particular reason for this order of joining but there is no other definite guide to assembling and the only criterion we have is the final result and its comparison with the type group. After this method of procedure had been followed all the chromosomes were rearranged, as in the first method, according to the lengths derived from the combinations. When this was accomplished the chromosome pairs stood out much more clearly than in the rearrangement by the first method. A graphic illustration of the results is given in plate 1, figures 4 a to 9 a. The same cells have been used for regrouping as in the first case. It will be noticed that the sudden "jumps" that are present between the chromosomes arranged by the first method are not evident between the chromosomes handled by the second method. The difference in length between the pairs which result from the second method is reasonably constant and approaches more nearly the

length relations of the type group. In table 2, as in table 1 the numbers opposite the chromosome pairs refer to the length in centimeters and the number between any two pairs gives in percent the length of the shorter pair in comparison to the next longer pair.

It is evident from table 2 that there is relatively small variation, not only in the average percent but between the individual pairs. As was pointed out earlier, the variation in some cases between individual pairs by the first method is quite high and there is little uniformity in the percentage of variation. Between the pairs of the type group exists a fairly uniform difference of nine percent. This condition must be approximated in the results of reuniting the broken chromosomes and this is much more nearly accomplished by the second method than by the first. In the table dealing with the first method (table 1) are to be found instances where this difference rises to 22, 24, and 26 percent, and several cases of variations of 14 and 15 percent. In the second table 12 percent is the greatest length difference recorded and this is not frequent.

TABLE 2

Pairs	Total number of chromosomes						Average percent
	16	17	18	19	20	21	
AB	6.4	6.4	6.1	5.8	6.1	6.3	
	89%	90%	88%	88%	88%	90%	89%
CD	5.7	5.8	5.4	5.1	5.4	5.7	
	96%	93%	96%	94%	92%	91%	94%
EF	5.5	5.4	5.2	4.8	5.0	5.2	
	92%	94%	94%	90%	96%	94%	93%
GH	5.1	5.1	4.9	4.3	4.8	4.9	
	94%	90%	94%	95%	96%	88%	92%
IJ	4.8	4.6	4.6	4.1	4.6	4.3	
	94%	96%	94%	95%	91%	93%	94%
KL	4.5	4.4	4.3	3.9	4.2	4.0	
	96%	93%	91%	98%	95%	100%	95%
MN	4.3	4.1	3.9	3.8	4.0	4.0	
	88%	90%	92%	90%	100%	95%	92%
O	3.8	3.7	3.6	3.4	4.0	3.8	
Average percent	93%	92%	92%	93%	92%	93%	

c. *Discussion of the methods of analysis and of the results obtained with them*

An interesting and at first seemingly contradictory relation becomes apparent when an average of the percentage relationships between pairs

for all groups and by both methods is made and compared with the same figures derived for the type cells.

There is apparently very little difference between the two methods as far as the average percentage of variation is concerned. The average of the percentage of length variation, however, does not give a true conception of the conditions. The average in this instance hides rather than reveals the facts and recourse must be made to the complete tables in which the individual relationship between pairs is figured. Only then can it be appreciated that this relationship is much more constant and uniform in the rearrangements by the second method. That the *general average* of the percentage of length relationship should be about the same in each case seems at first anomalous. It is easily understood, however, when it is recalled that *the amount of chromatin in a cell* (however much fragmented it may be) *remains the same.* Since this is true any method of reuniting the chromosomes would give about the same *average* result because *the total amount of length differences must always be the same*

TABLE 3

The length relation of the chromosome pairs in percent

Pairs	Average of type group	Average of all groups	
		First method	Second method
AB	91%	89%	89%
CD	93%	93%	94%
EF	91%	88%	92%
GH	94%	89%	93%
IJ	95%	91%	94%
KL	89%	86%	95%
MN	88%	86%	92%
O			
Average	91%	89%	92%

regardless of how it is distributed and providing no chromatin is lost. The real criterion, therefore, can only be the pairing and the uniform relation between *individual* pairs.

It might be pointed out further in objection to the first method that the comparison of the chromosomes in any column with the type minimum for that column will, in the majority of cases, be of little value as a criterion for discovering a chromosome that has lost a portion of its length. As stated under the description of the second method, the fragmented chromosomes will in the greater number of cases lie at the foot of the series with a consequent displacement of the short chromosomes. These then must in all probability fall in columns where they will be even shorter than the type minimum for that column. The first method considers these as fragmented chromosomes when in reality they are probably the shorter chromosomes displaced. This objection does not hold for the second method.

A second criticism that may be made of the first method, for the present work, is that there is a tendency to build up the long end of the series to the maximum length, since, as a rule, longer chromosomes are selected to which to add the fragments. This will become clearer when it is recalled that the chromosomes of the short end of the series are displaced forward through the fragmentation of the longer chromosomes. These forwardly displaced chromosomes will, in all probability, be shorter than the type minimum for the column in which they fall and will be selected for mating with the fragments. These short chromosomes are, however, very probably of greater length than the true mates of the fragments (as demonstrated by the second method). Consequently the chromosomes united by the first method may be abnormally long (even though the type maximum is not exceeded) in comparison with the short end of the series. In brief one end of the series is developed at the expense of the other. This again is not true of the second method. It may be seen in the tables under the two methods that the short chromosomes fall very close to the type minimum for the same chromosomes in the first method and the long end of the series approaches the type maximum. In the second method the chromosomes are much better distributed in relation to the type average. Figure 3 illustrates this point for the first method. It will be seen that the light lines representing the chromosome lengths of the rearranged fragmented cells start well on the maximum side of the average and then fall until at the lower end of the series they are below the average and somewhat irregularly spaced. Figure 4 is a similar graph for the second method. It is evident that though few chromosomes start above the average they compare very favorably with it and follow it closely all the way. All the lines are much closer together

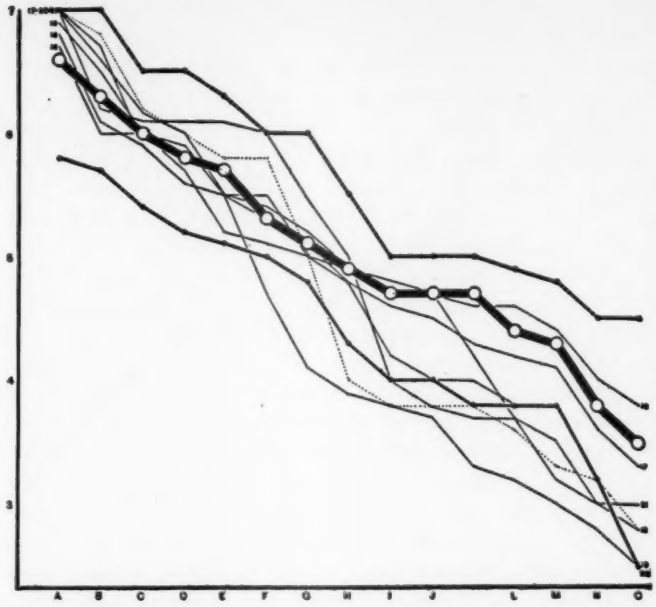

FIGURE 3.—The result of the recombination of the chromosome fragments by the first method. The letters along the base line refer to the chromosomes and the numbers on the axis of ordinates indicates length in centimeters (17,000 diameters). The heavy band represents the average length of the type chromosomes. The heavy lines above present the maximum chromosome length found in the type group, the corresponding curve beneath the average curve gives the minimum chromosome length found in the type group. The light curves between these lines represent the average length of the recombined and rearranged chromosomes for all of the examples on plates 6 and 7. These lines are numbered according to the group they represent. The dotted line represents not an average of a group but the lengths of the chromosomes of the "longest" cell in the 19 group. Note that these light lines start *above* the average curve and separate widely at the short end of the series.

than in the previous chart, indicating a much more nearly uniform length in each case. Even the "19" group, which falls below the minimum curve, as might be expected because of its shorter total length, is very slightly under this curve and on this graph can be seen to run closely parallel with the average.

Hence the second method of analysis produces better differentiated pairs which possess a more uniform relation to one another (such as exists between the pairs of the type group). Since it appears to be

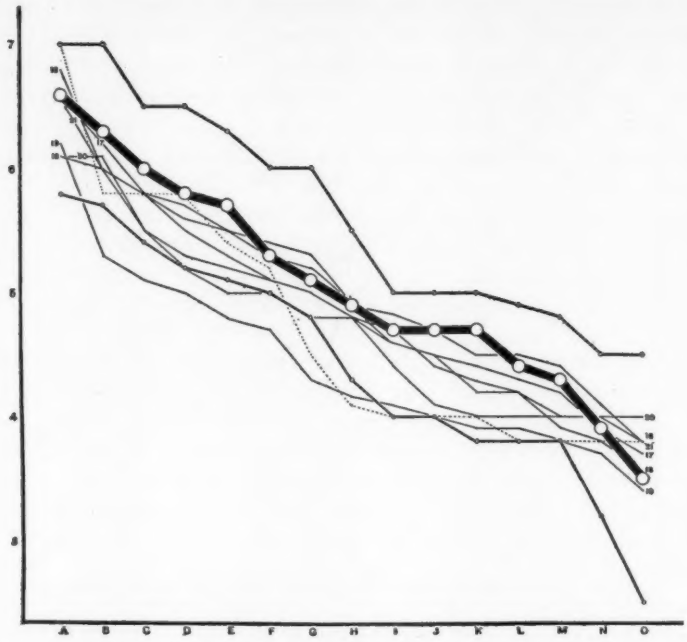

FIGURE 4.—Chart graphically illustrating the result of the recombination of the chromosome fragments by the second method. All lines, letters and numbers correspond to those in figure 3. Note that the light lines in this case remain more closely together than in figure 3 and are more nearly parallel to the curves of the type average.

generally more logical, I believe that it is the better method for this particular problem. The first method, however, will prove of greater value in the study and comparison of the cytological features of other Oenotheras where the chromosome number is constant. There are many Oenothera mutants possessing the same number of chromosomes as *Oe. Lamarckiana* and by applying the first described method it should be possible to determine whether the chromosomes are comparable in all cases or whether there may be size variations which may indicate the causes of the mutations. The many possibilities of one or both of the methods described need not be further developed as they will readily suggest themselves to the reader. The methods, of course, will apply to animals as well as to plants and in a recent study of the diploid chromosome complexes of the pig (HANCE 1917) I have obtained excellent results through the application of these methods to the analysis of the

chromosome peculiarities there found. If the entire methods are not of use to the investigator, measurements may often be a very convenient mode of determining whether or not all the chromosomes are present in forms possessing many chromosomes and in cases where it is suspected that fragmentation has taken place.

While it seems probable that in many or possibly the majority of the instances, the fragments may not have been properly restored, nevertheless the exactness with which the recombinations and rearrangements fall within the limits of the type group not only in length but also in difference in length between the pairs is very suggestive and would seem to indicate that the regrouping has been approximately correct.

We are now in position to answer the questions set at the beginning of this analysis, i.e., *is it possible to determine which chromosomes have fragmented and whether the same chromosomes are involved in all cases?* It is decidedly interesting to note that the chromosomes that have fragmented belong chiefly at the long end of the series—from A to H. There is some indication that the longer chromosomes tend to break up first as we find that nothing below H has fragmented in the "16" group, nothing below I in the "17" group and so on. This can be seen in the following table.

TABLE 4

The number of cases of reunited fragments whose combined length indicates that they fall below "H", the eighth chromosome, in the length series

Cell groups	Chromosomes			
	I	L	N	O
16				
17	1			
18		1		
19	1	1	1	1
20	1			
21	1			

The fact that a set of joined fragments falls in the "O" column in one case, indicating that chromosome O has fragmented, is borne out in one cell by observational data. The unpaired chromosome O in row 1, plate 6, shows a marked constriction which indicates an example of fission. I have at present no explanation for the fragmentation of the longer chromosomes.

THE CHROMOSOMES OF THE HETEROTYPIC DIVISION

One of the best stages for determining the haploid number of chromosomes is during interkinesis following the first or heterotypic division in the pollen mother cell. At this time the individual chromosomes show a split anticipating the second or homotypic division and open into X-like structures.

a. The classes of gametes

Two types of telophase nuclei are formed by the heterotypic mitosis —one with seven chromosomes (plate 5, figure 23) and the other with eight (figure 25, plate 5). Consequently there are finally developed two kinds of gametes, those possessing seven chromosomes and those with eight chromosomes. Side views of the spindles of the heterotypic mitoses at metaphase give the explanation for the two classes of gametes as in all cases one chromosome was found without a mate and moving to one pole undivided (plate 5, figures 21 and 22). The division shown in these figures of the odd chromosome is the split referred to above which anticipates the second mitosis. Hence one daughter nucleus receives one more chromosome than does the other. Figure 24, plate 5, an anaphase of the heterotypic mitosis, shows one chromosome lagging behind the others of the two groups. GATES and DAVIS have reported for some of the Oenotheras that such chromosomes may remain behind in the cytoplasm, and similar conditions have been noted in other plants.

A point of considerable importance is indicated by the study of the germ cells. Fragmentation of the chromosomes, as far as has been observed, apparently does not occur in the nuclei of the germinal line, but is confined to the soma.

b. The measurement of the chromosomes during interkinesis

Each split chromosome (the X-like structure) in the stage of interkinesis should represent, according to current theories, one member of each pair of the somatic complex. The secondary split present at this stage does not affect the problem of the measurement of the chromosomes. Since these chromosomes are evidently shorter than the somatic chromosomes it was thought well to determine, if possible, whether the individuals fell into a graded series with a definite size relation between them as did the pairs of the somatic cells.

In the following table the lengths, as before, are listed in centimeters. The figures for the seven-chromosome class are an average of four cells and those for the eight-chromosome class an average of six cells. While these are small numbers, on which to base measurements of this sort, the chromosomes were particularly well shown and I believe the results give a fairly accurate expression of the facts.

TABLE 5

Lengths of the chromosomes in the two classes of nuclei

	1 A or B	2 C or D	3 E or F	4 G or H	5 I or J	6 K or L	7 M or N	8 O	Total length
7 chromosomes	4.8	4	3.9	3.5	3.3	3	2.7		25.2
8 chromosomes	4.5	4.1	3.7	3.6	3.3	3.1	2.8	2.6	27.7

This table shows that the chromosomes of the period of interkinesis fall into a graded series. The pairs that they theoretically represent are placed beneath the numbers indicating the series. As would be expected the total lengths of the chromosomes in the two classes are not alike, the figure for the class possessing the extra chromosome being larger. If the first seven chromosomes of the eight group are added the result is 25.1 centimeters which is just one millimeter shorter than the length given for the seven group. Consequently the gametes may be said to differ as far as total chromosome lengths are concerned by the length of the extra chromosome 8. It seems evident from the lengths of the chromosomes in the two types of gametes that all of the corresponding chromosomes (chromosomes 1 to 7) are nearly enough of even length to be considered as mates. This leaves chromosome 8 out as unpaired and since it is also the shortest chromosome of this series it seems reaonable to consider this to be chromosome O of the somatic complexes.

It can now be seen that the chromosomes 1 to 7 are comparable to one or the other member of the somatic pairs AB to MN and that chromosome 8 is chromosome O. The chromosomes of the reduction division are much shorter but they are also wider and it is believed that the amount of chromatin is the same. If the individual relationship is similar the evidence in favor of the supposition that the chromosomes 1 to 8 are definitely related to the somatic chromosomes would be strong.

Table 6 shows that the relationships between the individual chromo-

TABLE 6

Comparison of relationships

Somatic pairs	Length percentages of pairs	The chromosomes of the interkinesis	Length percentages in 7-chromosome nuclei	Length percentages in 8-chromosome nuclei
AB	91	1	83	90
CD	93	2	98	90
EF	91	3	90	97
GH	94	4	94	92
IJ	95	5	91	94
KL	89	6	90	92
MN	88	7		93
O				
Average	91		91	92

somes of the two classes of gametes is remarkably close to the relationships between the pairs in the somatic type group.

The average length of chromosome 1 of the classes 7 and 8 is 4.7 cm. This is 72 percent of the average length of the somatic pair AB. Since the variation between the heterotypic chromosomes is relatively constant, the multiplication of the lengths of the chromosome pairs AB to MN by 0.72 should give approximately the length of the corresponding germ chromosomes.

TABLE 7

Somatic pairs	Length of somatic pairs	Multiplied by	Result	Average of observed measurements
AB	6.4 cm	0.72	4.7 cm	4.7 cm
CD	5.9 cm	0.72	4.2 cm	4.1 cm
EF	5.5 cm	0.72	4.1 cm	3.8 cm
GH	5.0 cm	0.72	3.6 cm	3.6 cm
IJ	4.7 cm	0.72	3.4 cm	3.3 cm
KL	4.5 cm	0.72	3.2 cm	3.1 cm
MN	4.0 cm	0.72	2.9 cm	2.8 cm
O	3.5 cm	0.72	2.5 cm	2.6 cm

When these *calculated* lengths (second column from right) are compared with the first column on the right giving the averages of the *observed* measurements it will be seen how close to one another are the two sets of figures.

DISCUSSION

a. A comparison of certain somatic chromosome studies

Variation in somatic chromosome number has been described by several writers, but the reports have never carried complete conviction, since (1) the results were so contrary to those constantly obtained in the germ cells of many plants and animals, (2) the connection between the "extra" chromosomes and those of the germ cells was not shown and (3) the work was frequently open to a well justified criticism of the technique. If variation in somatic chromosomes occurs the investigator must be able to meet the above criticisms if his work is to stand.

In a recent review of the work done on the somatic chromosomes of animals, HOY (1916) summarizes the reported conditions as follows:

"In considering the foregoing cases it is apparent that in general the behavior of the chromosomes of the somatic cells can be classified in the manner stated in the introduction of this paper, namely that (1) the somatic and gonial chromosomes agree in number and type, (2) the number of chromosomes in the somatic cells is higher than in the germ cells, due to a splitting or fragmentation, (3) multiple chromosome groups are produced by the suppression of cell division, and (4) there is a synapsis, more or less permanent of all or certain pairs of the chromosomes in each cell generation. It is also evident that there are numerous cases which are exceedingly perplexing, and which cannot be classified at the present time."

HOY also writes:

"If there may be variation in the number of chromosomes in the various cells of the same individual, it is certainly not of general occurrence, and no really clear case has been presented. I believe that variation in number is more often between individuals of the same species than between cells and tissues of the same animal. Variations in the size of the chromosomes are more difficult of analysis, and more difficult of demonstration."

The present study offers a clear case of variation in chromosome number between the cells of an individual, and an equally clear case is described in my paper (HANCE 1917 c) on the diploid chromosome complexes of the pig.

I am in agreement with HOY that the work on the variable numbers of

chromosomes is unsatisfactory, as far as animals are concerned. A great many chromosome counts are untrustworthy because the material was not properly prepared. This is particularly true of the work on mammalian chromosomes, and in a recent paper (HANCE 1917 b) I have shown the effect of different methods of fixation on the chromosome numbers in mammals. An account of the multiple complexes in the mosquito, *Culex pipiens,* derived through chromosome multiplication, has recently been published (HOLT 1917). The number ranges from nine to seventy-two although the normal count is six. While this is doubtless atypical tissue the results are exceedingly important since they tend to show the effect of certain conditions on the cell. In pig embryos (HANCE 1917 c) I have found variable numbers of chromosomes, the results of fragmentation as in *Oenothera scintillans.*

Since HOY has thoroughly reviewed the work on somatic chromosomes of animals I shall consider only a few cases that have some bearing on the present work. Very little work has been done on measuring chromosomes and with the exception of papers of MEEK (1912) and FARMER and DIGBY (1914) there are to my knowledge few other published accounts. In the studies of FARMER and DIGBY the part of greatest interest for the present problem is the statement that the

"total amount of chromatin substance in the nucleus of each of the two types of hybrids known as *Primula kewensis* is the same. The nuclei of one form of hybrid contains twice as many chromosomes as the nuclei of the other type, but the increase in number is associated with a corresponding diminution in size."

It would have been more satisfactory if the authors had published their methods of measuring. It is interesting, however, to note that a result apparently similar (as far as length is concerned) to the present work was obtained on a form differing in chromosome number. MEVES (1911) found a graded series in the salamander although the exactness of the seriation did not seem to impress him. He measured the chromosomes with a toothed wheel.

FRASER and SNELL (1911) have found in the kidney bean, *Vicia faba,* that the chromosomes on the spindle were frequently made up of two or more distinct segments and suggest that these segments may be discrete units capable of separating or uniting, thus increasing or diminishing the number of chromosomes. While the constrictions are similar to those found in *Oe. scintillans,* no cases of fragmentation were reported.

GATES and Miss THOMAS (1914) have shown that *Oe. lata* has fifteen chromosomes. In the reduction divisions, as in the case of *Oe. scintillans,*

one chromosome goes to one pole undivided thus producing two types of gametes. There may be some variation in this process. GATES is inclined to believe that the undivided chromosome bears a resemblance to the "accessory" chromosome in animals which is associated with sex, if, indeed, it is not sex-determining. In the case of *Oe. lata* GATES (1915, p. 181) suggests that "a *lata*-like mutant is produced in the presence of a triplicate for a certain pair of chromosomes." Since he developed no methods for analysing his data he very naturally concluded that

"At present it appears probable that the results will be essentially the same, to whichever pair of chromosomes the extra one belongs. But it is conceivable that seven types of such mutants might occur, as each of the gametophytic chromosomes may constitute the extra one."

With the methods here described for the analysis of the chromosomes of *Oe. scintillans* it is possible to show rather clearly that the "extra" chromosome in the eight-chromosome gametes was the unpaired chromosome O and no other.

I was interested to know what would be the results of the application of the methods employed in this study of *scintillans* to the work of other investigators of Oenothera cytology. While I was writing this report, the appearance of two papers by MISS LUTZ (1916, 1917) on Oenothera chromosomes presented a good opportunity for the application of my methods to her data. These cases are of special interest since Miss LUTZ describes a "diminutive chromosome" suggestive of fragmentation in some variants from 14-chromosome Oenotheras.

Accepting the chromosomes as they are figured by Miss LUTZ regardless of the question of fixation (see page 228 in this paper) it was interesting to determine the relation of the chromosomes to each other and to compare them with those of the present study. The length measurements are probably reliable since when shrinkage in length occurs I have found that it apparently affects all chromosomes in the same degree. Three cells of *Oenothera aberrans* (LUTZ 1916, figures 1, 2 and 3), were chosen for comparison and Miss LUTZ's drawings of the chromosomes have been reproduced in figure 5 with the chromosomes separated and arranged in a series according to length. Since the difficulties of enlarging from published figures are sufficient to admit of considerable error, only approximate accuracy is claimed for the following results. In her studies on the somatic chromosomes of the Oenotheras Miss LUTZ has reached the following conclusions.

1. She agrees with GATES that the chromosomes of the Oenotheras

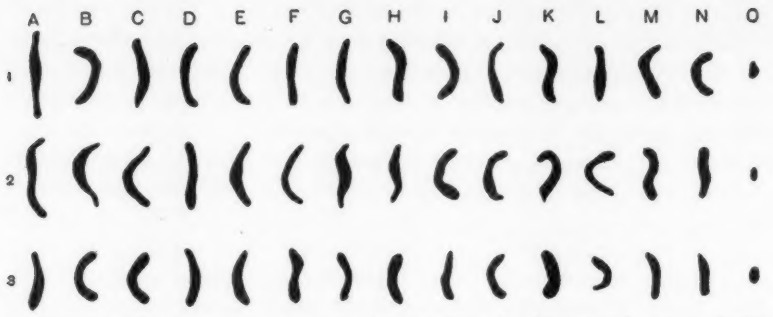

FIGURE 5.—Chromosomes of three cells presented in Miss Lutz's paper (1916, figures 1, 2 and 3), separated and arranged. It is evident that a graded series exists and that the chromosomes in each column are approximately equivalent in length indicating a uniform and constant size relation.

present no constant difference in size and shape with the exception of the small chromosomes of the 14^{+1}-chromosome mutants.

2. She suggests that the "diminutive" chromsome is a portion of a "half chromosome" (GATES's term) of the germ cells which, having degenerated, has left this fragment in the cells of the 14^{+1} plants. She apparently favors, however, the view that the small chromosome has been derived by fragmentation of one of the longer chromosomes.

3. The "diminutive" chromosome supports the theory of the individuality of chromosomes, as it is constant in size.

The chromosomes of the three figures of *Oe. aberrans* in the metaphase of mitosis (LUTZ 1916, figures 1, 2 and 3) were enlarged six diameters and measured. The length relationships between the chromosomes in these cells are given in table 8.

It is quite evident from this table and from figure 5 that a series of

TABLE 8

Length of the chromosomes in Miss LUTZ's *figures, in centimeters*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
Figure 1	4	3.8	3.4	3.2	3.2	3	3	3	3	3	2.9	2.8	2.8	2.3	0.7	44.1
Figure 2	4.2	3.6	3.6	3.2	3.2	3.1	3.1	3	3	3	3	3	2.6	2.3	0.5	44.4
Figure 3	3	3	2.9	2.9	2.8	2.8	2.5	2.5	2.5	2.5	2.3	2.3	2.1	2	0.7	36.8

chromosomes grading from long to short, exists in *Oe. aberrans* as in *Oe. scintillans* and furthermore that the lengths are constant for particular chromosomes. In the case of her figure 3 all the chromosomes are shortened proportionally (possibly due to fixation) as is shown in the percentage relationships of the pairs. Since the chromosomes are broader the same amount of chromatin is believed to be present. The percentage relationships existing between the chromosome pairs of these cells are given in table 9.

TABLE 9

Pairs	Figure 1	Figure 2	Figure 3
1-2	3.9	3.9	3.0
	84%	87%	97%
3-4	3.3	3.4	2.9
	94%	94%	78%
5-6	3.1	3.2	2.8
	97%	97%	89%
7-8	3.0	3.1	2.5
	100%	97%	100%
9-10	3.0	3.0	2.5
	97%	100%	92%
11-12	2.9	3.0	2.3
	90%	83%	91%
13-14	2.6	2.5	2.1
Average percent	92%	93%	91%

It is apparent that there exists approximately the same difference between the pairs in this case as in the case of *Oe. scintillans* and this difference is as constant in most cases as can be expected considering the difficulties involved in making the measurements. There is apparently one pair in each cell which is enough shorter than the next pair, to cause its percentage relationship to differ quite markedly from the other percentages. In Miss LUTZ's figure 1 this is pair 3 and 4, in her figure 2 it is pair 13 and 14, and in her figure 3 either pair 3 and 4 or 5 and 6. When the fragment is added to one of the chromosomes and these chromosomes rearranged according to the new lengths it is found that the relationship of the pairs is slightly improved. The table below gives the results of the rearrangements. In her figure 1 the "diminutive chromosome" has been added to chromosome 7, in figure 2 to chromosome 14 and in figure 3 to chromosme 3 (see table 10).

It must be admitted that the demonstration in this case with respect to the percentages obtained after rearrangement is not as striking as in *Oe. scintillans*. I believe, however, that the results obtained with *Oe.*

TABLE 10

Pairs	Figure 1		Figure 2		Figure 3	
1-2	3.9		3.9		3.3	
		87%		92%		91%
3-4	3.6		3.4		3.0	
		89%		94%		93%
5-6	3.2		3.2		2.8	
		94%		97%		89%
7-8	3.0		3.1		2.5	
		100%		97%		100%
9-10	3.0		3.0		2.5	
		97%		100%		92%
11-12	2.9		3.0		2.3	
		90%		90%		91%
13-14	2.6		2.7		2.1	
Average percent		94%		93%		92%

aberrans are as close to those of *Oe. scintillans* as can be expected in view of the possibilities of technical error, and are exceedingly suggestive. Whether the results of the reuniting of the supposed chromosome fragments are accepted or not it is very plain that *the chromosomes are constant as to length* and that a graded series exists. This is strong evidence in support of the theory of individuality. If the logic of the reuniting of the fragments is admitted it is evident that the small chromosome is not the same body in each cell and is apparently derived from a different chromosome in each instance. The "diminutive chromosome" of Miss LUTZ therefore appears to me best interpreted as a fragment of uncertain derivation and cannot be claimed as constant in origin. This would, of course, modify its value as evidence for the hypothesis of chromosomal individuality.

b. The individuality of chromosomes

Since no chromatin appears to be lost through the process of fragmentation in the soma of *Oe. scintillans,* and as the fragments divide as regularly as before their separation, it would seem that the chromosome fragments maintain their morphological identity. If the theory of the individuality of chromosomes recognizes only a strict morphological continuity then in *Oe. scintillans* many chromosomes lose their individuality through fragmentation. If, however, the theory be extended to admit an individuality not only of whole chromosomes but of fragments as well then the *scintillans* situation gives to the theory strong support. I believe cytologists are tending toward the latter interpretation today. I have

been able to show quite conclusively that the length of the chromosomes in *Oe. scintillans* is constant, that there is a very definite pairing of the chromosomes, that the fragments may be so combined as to approximate the type conditions, and that the somatic chromosome complex is comparable and related to the chromosomes of the germ cells. With these data we are fully justified in claiming that the chromosomes possess individuality.

The small difference in the size of Oenothera chromosomes which makes optical differentiation difficult, has led to erroneous conclusions such as the following which has been extracted from Miss LUTZ's paper on diminutive chromosomes in Oenothera mutants (LUTZ 1916).

"GATES found that the chromosomes of Oenothera present no constant differences in size and shape, and with the exception of the small chromosome in the 14^{+1}-chromosome mutants, the observations of the writer are in full accord with those of GATES. The small member of the 14^{+1}-chromosome group, however, presents a very conspicuous exception, for we have seen that it may be recognized constantly by its shape and by its diminutive size. It furnishes very strong evidence in support of the theory of chromosomal individuality."

Why one chromosome should support the individuality hypothesis when the chromosomes from which it presumably has arisen "present no constant differences in size and shape" is rather hard to see. I have already analysed (page 251) the chromosomes of three cells reproduced in Miss LUTZ's paper and have been able to show that *the chromosomes of these cells are very constant in size and shape* and consequently do support the theory of individuality. The evidence seems rather to favor a view that the diminutive chromosome (fragment) may have been derived from a different member in each case and consequently does not support the individuality hypothesis since it is not the same body in each instance.

c. The cytological basis of the genetics of Oenothera scintillans

In connection with the cytological study of *Oe. scintillans* its genetic behavior is of interest and importance since it may possibly be understood on the basis of chromosome distribution. *Oenothera scintillans* is one of the unstable mutants from *Oe. Lamarckiana* and DEVRIES (1916) has recently reported three new mutants which behave similarly. When selfed, *Oe. scintillans* never breeds true, but throws several forms in varying percentages. DEVRIES thinks that the capacity to produce these various forms is inherited, since a line of *Oe. scintillans* usually throws similar percentages of the same unlike types. In table 11 are given some

of the results obtained by DEVRIES (1909) from selfed plants of *Oe scintillans*.

TABLE 11

Oe. Lamarckiana	68	55	(52–59)	51	8
Oe. scintillans	15	37	(34–36)	39	69
Oe. oblonga	15	7	(3–10)	8	21
Oe. lata	2	1	(1)	1	2

Oe. nanella is occasionally produced.

The following percentages (table 12), obtained from DAVIS's records of selfed *Oenothera scintillans* for the last three years, although based on smaller numbers are very probably more accurate than those of DEVRIES, since they are based upon a *total* germination of the seeds. DEVRIES's figures were gathered from seeds sown in the earth while the results recorded below are of plants from seeds germinated according to the methods recently described by DAVIS (1915 a), and are from cultures in which germination was experimentally proved to be complete.

TABLE 12

	Date	Percent	Number of plants	Complete germination
scintillans	1915	11	4	33
oblonga		8	3	
Lamarckiana		81	30	
scintillans	1916	11	6	33
oblonga		8	3	
Lamarckiana		65	33	
scintillans	1917	10	6	33
oblonga		8	3	
Lamarckiana		69	40	

Average percentage for the total of 146 plants:

scintillans	11
oblonga	19
Lamarckiana	70

Since there are two classes of gametes produced in *Oe. scintillans* the zygotes resulting from self-fertilization might be expected to be of three types. The reason for this is explained in the following combinations:

A 7-chromosome gamete uniting with another 7-chromosome gamete would give a 14-chromosome zygote.

A 7-chromosome gamete uniting with an 8-chromosome gamete would give a 15-chromosome zygote.

An 8-chromosome gamete uniting with an 8-chromosome gamete would give a 16-chromosome zygote.

Since *Lamarckiana* has 14 chromosomes, and *scintillans* and *lata* 15 chromosomes, the appearance of these plants from cultures of selfed *scintillans* is as might be expected. The chromosome count in *Oe. oblonga* from *scintillans* has not yet been made. Should it prove to be 16 chromosomes there would be presented a type for each gametic combination.

It is quite evident that *scintillans* characters are at least associated with chromosome O if this chromosome does not actually produce them. This situation calls into question GATES's suggestion that the mere presence of an extra chromosome produces a *lata*-like mutant, since in this case there is also an extra chromosome in a *scintillans* plant. It appears probable that either there is considerable differentiation among the chromosomes of Oenothera or that other factors in the cell are producing the same cytological situation as regards the number of chromosomes (15) in both *scintillans* and *lata*, although these two plants differ markedly in their specific characters. The former condition would seem more probable in view of our general knowledge of chromosomes and of the evidence presented by the two plants themselves.

With the above facts before us the explanation that immediately suggests itself is that the variability of the somatic chromosome number in *Oe. scintillans* is either merely an associated coincidence or is an expression of its genetic instability. Two views of the Oenothera situation are argued by DEVRIES and DAVIS, respectively, the former maintaining that *Oenothera Lamarckiana* is a pure species and that the new forms thrown by it are "mutants," the latter holding the view that the original plant is not genetically pure and that the new types are the expression of its hybrid organization. Cytologically the evidence would seem to favor the "hybrid" hypothesis since many of the cytological peculiarities reported for the Oenotheras, such as the lagging of chromosomes and their failure to be included in the reformed nucleus, failure to pair regularly in the heterotypic mitosis, irregularities in the distribution of the chromosomes, and the partial or total sterility of the gametes and zygotes are conditions known to be common in the germ cells of hybrids. In a race of *Oe. grandiflora*, studied by DAVIS (1909), where the pollen sterility is very low, the chromosomes of the heterotypic division are definitely paired and present an orderly arrangement such as is found in pure species. DAVIS (1915 c) has used the results of his studies on *Oe grandi-*

flora very effectively in his criticisms of the purity of *Oe. Lamarckiana*, in which the chromosomes are not regularly paired in the heterotypic mitosis. Of the various forms that result when *scintillans* is selfed, *scintillans* and *lata* have 15 chromosomes, and we do not yet know the count in *oblonga*. The relation of the chromosomes of these forms to each other and to those of *Lamarckiana* will prove a most interesting cytological investigation, a study for which the methods of analysis employed in the present work are particularly adapted. It seems very probable that the main problem will be the determination of the identity of the extra chromosome when present. It also appears probable that the extra chromosome will be a specific structure for each of the forms considered.

There are cytologists who will probably seriously object to the reduction of the study of chromosomes to mathematical formulae. It remains only to be pointed out to these workers the definiteness and accuracy with which the extra chromosome in *scintillans* has been determined in comparison with the results obtained on the odd chromosome of *lata*. To this may be added the clearness with which the paired relation of the somatic chromosomes in the type group has been brought out, the relationship of the pairs, the analysis of the fragmenting cells with the successful regrouping of the chromosomes and lastly the relation and similarity of the germinal and somatic chromosomes, all of which would have been impossible to determine except through the methods employed. Our only means of studying the vital activities of the chromosomes at present is through morphological comparisons and since there are few plants or animals which have chromosomes sufficiently well differentiated to be recognized by characteristic shapes alone other methods must be resorted to. It is hoped that the measuring of the chromosomes and the subsequent use of the methods of analysis developed in this paper may have a wide application in cytological investigation and that through them a better and more exact knowledge of the morphology of the chromosomes and of their behavior may be obtained.

SUMMARY

1. In material collected from three generations of *Oenothera scintillans* it has been found that the somatic chromosome number varied from 15 to 21, (plates 2 and 3, figures 1 to 12). This variation was not correlated with any particular tissue but existed throughout the same plant. The percentage of the different classes of variants is approximately the same for the three generations.

2. The type or fundamental number of chromosomes for *Oe. scintillans* is 15.

3. There have been counted in the anaphases 15 and 16 chromosomes and it is believed that all the chromosomes in the higher counts divide regularly (page 232, and plate 4, figures 14, 18, 19 and 20).

4. When the chromosomes of the type group are measured and arranged in a series in respect to length it is found that definite pairs exist and that there is also present a very constant length difference between the pairs. There are seven pairs and one unpaired body designated chromosome O.

5. Each somatic pair of the type group differs from the next in the series by about nine percent, i.e., a shorter pair is about ninety-one percent of the length of the next longer pair. This is apparently a constant relation.

6. The additional chromosomes above the type number (15) are believed for the following reasons, to be produced through the fragmentation of the type chromosomes.

a. Chromosomes are found in stages of constriction indicating points at which fragmenting may take place (plates 2 and 3, figures 1, 3 and 4, and plate 3, figures 9, 10 and 11).

b. The sum of the lengths of the chromosomes is the same whether the number be 15, 16, 17, 18, 19, 20 or 21 (see figure 2, page 235).

c. The differences between adjacent chromosome pairs in the classes other than the type group are not constant as they are in the type group, indicating that the length relations have been disturbed.

d. In the classes with additional chromosomes those that fall below chromosome O (the fifteenth chromosome) in the length series, are considered as fragments. When these fragments are joined to certain chromosomes lying between chromosomes A and O by the method described in the text, and when the chromosomes are rearranged according to the new lengths, it is found that the pairing is much more obvious and that the new length relations of the pairs is practically identical with those existing in the type group (see plate 1, page 263).

It is believed that these four points prove conclusively that fragmentation of the chromosomes takes place in *Oenothera scintillans*.

7. Fragmentation has been confined largely, but not exclusively, in the cells studied, to the longer chromosomes.

8. Fragmentation has not yet been observed in the cells of the germinal line.

9. Two classes of gametes are found, one possessing 7 chromosomes and the other 8, since the unpaired chromosome O in the first or heterotypic (reduction) division passes to one of the poles (plate 5).

10. The 8-chromosome class differs in the total length of the chromosomes from the 7-chromosome class by the length of the eighth chromosome (the equivalent of the somatic chromosome O).

11. It has been shown that the same length relations exist between the individual chromosomes of the haploid group as between the somatic pairs and that these two sets of chromosomes are therefore related. It has been demonstrated that the eighth chromosome of the haploid group is the unpaired chromosome O of the somatic complex (page 247).

12. The data presented above has been used as a basis for the following suggestions.

a. The individuality of the chromosomes of *Oe. scintillans* is not destroyed by fragmentation, however much their morphology may be modified (page 254).

b. Chromosome O is correlated with *scintillans* characters, if it is not actually the *scintillans* determiner (page 257).

c. Chromosome O is an unpaired chromosome but has no association with sex as is usually attributed to the odd chromosome of the germ cells of animals (page 251).

d. The two classes of gametes explain the genetic behavior of *Oenothera scintillans* (page 256).

13. It is believed that the methods of procedure adopted in this study will be found applicable to a number of cytological problems, since they afford a means of determining certain chromosome relations more accurately than is possible through purely observational study and also because they permit the investigator to eliminate largely the personal element from his results by supplying him with a number of mechanical and mathematical checks.

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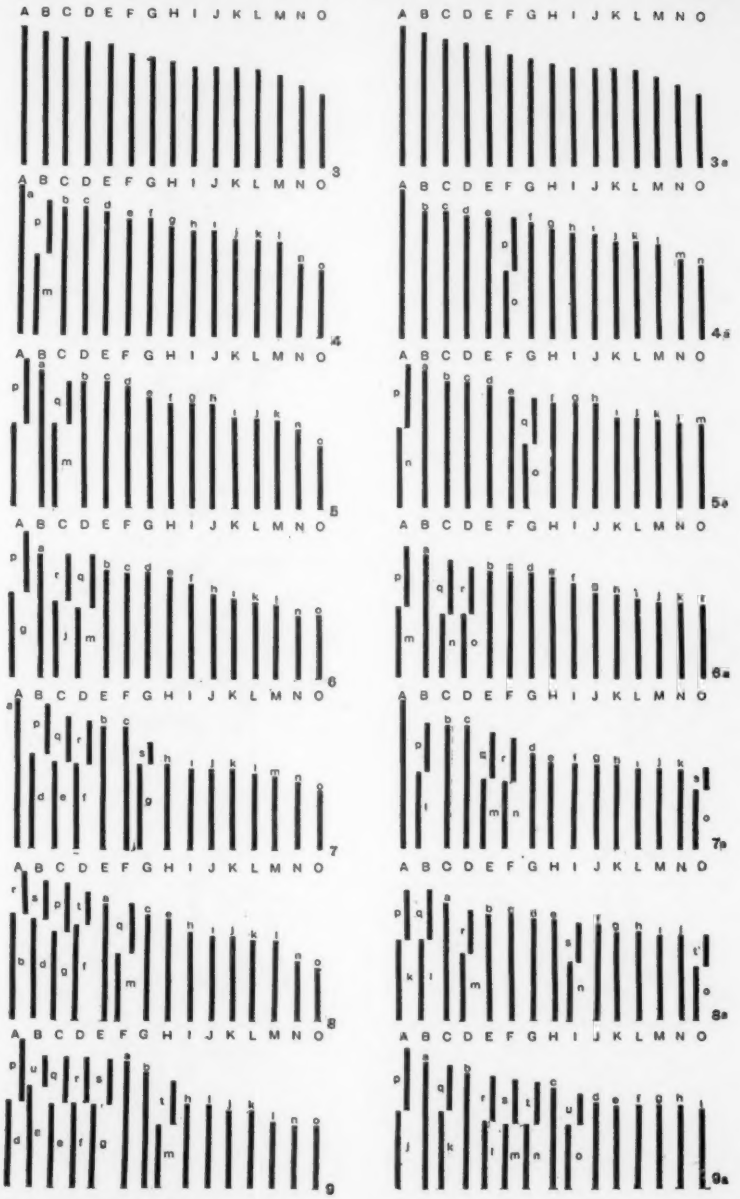
EXPLANATION OF PLATES 2 TO 5

The figures on plates 2 to 5 have been drawn at table level with the aid of a camera lucido. A Spenced 1.8 mm objective and a 12 X Zeiss compensating ocular were employed, which gave, at the level of the table, a magnification of 2800 diameters. The drawings were subsequently enlarged 3 diameters with a pantograph and reduced two-fifths in reproduction. The reproduced drawings present the chromosomes at 5040 magnification. Figures 1 to 20 illustrate somatic chromosomes, Figures 21 to 25, chromosomes of the heterotypic mitosis.

EXPLANATION OF PLATE I

Figures 3 and 3a are the same, representing the average length of the type chromosomes A to O. Figures 4 to 9 graphically represent the recombination and rearrangement of the chromosome fragments (drawn to scale) by the first method, 4a to 9a by the second method. In the recombinations the small letters indicate the columns the chromosomes or fragments occupy on plates 6 and 7. Note in figure 3 that the chromosomes form a graded series. The curve formed by a line joining the tops of the columns representing the chromosomes, approach the type curve more closely in figures 4a to 9a than they do in figures 4 to 9. The joined fragments lie most frequently at the long end of the series. The corresponding sets of drawings are based on data given in the description of plates 6 and 7,

figure 4 corresponding with plate 6, row 7,
" 5 " " " 6, " 12,
" 6 " " " 7, " 19,
" 7 " " " 7, " 20,
" 8 " " " 7, " 23,
" 9 " " " 7, " 24.



EXPLANATION OF PLATE 2

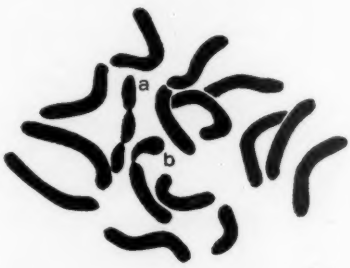
FIGURES 1 and 2.—Examples of the "type" or 15-chromosome group.

FIGURES 3 and 4.—16 chromosomes are present in each of these plates. Note the segmented chromosomes at *a* and *b* in figure 3. In figure 4 at *a*, *b*, *c* and *d* are to be seen chromosomes which show spaces relatively clear between dark chromatin granules. In the text it has been suggested that it is at these clear spaces that constrictions occur, followed by the separation of the parts.

FIGURES 5 and 6.—Examples of the 17-chromosome groups.

1

2



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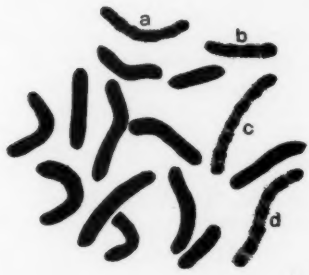

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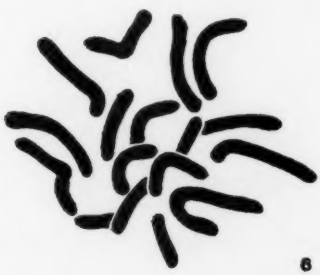


6

EXPLANATION OF PLATE 3

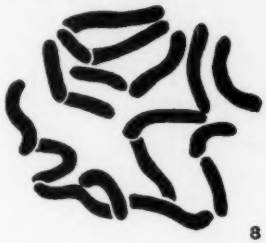
FIGURES 7 and 8.—Metaphase plates possessing 18 chromosomes.

FIGURES 9 and 10.—Metaphase plates showing 19 chromosomes. In both figures are shown at *a* instances where constrictions have apparently broken through, separating the two parts, although as yet these parts have not become widely separated. Figure 10, *b* and *c*, indicate metaphase chromosomes in which division is beginning.

FIGURE 11.—A 20-chromosome group. *a*, *b*, and *c* indicate chromosomes that have undergone fragmentation and the members are still aligned.

FIGURE 12.—A 21-chromosome group. The fragmented chromosome *a* shows its parts still in close relation to one another. Note the obvious difference in length of the chromosomes in this figure and of those in figures 1 and 2, plate 2.

7



8

9

10

11

12

EXPLANATION OF PLATE 4

FIGURE 13.—15 chromosomes present.

FIGURE 14, *a* and *b*.—The two poles of an anaphase figure with 15 chromosomes at each pole.

FIGURE 15.—17 chromosomes present.

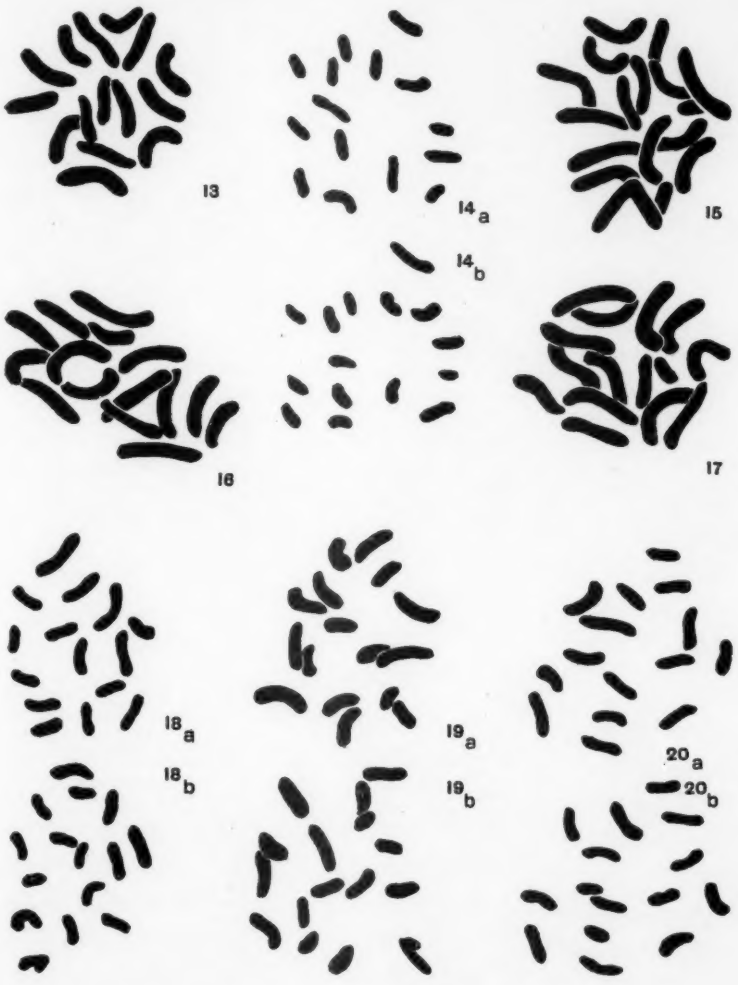
FIGURES 16 and 17.—15 chromosomes are in each of these plates.

FIGURE 18, *a* and *b*.—An anaphase figure with 15 chromosomes at each pole.

FIGURE 19, *a* and *b*.—An anaphase figure with 16 chromosomes at each pole.

FIGURE 19, *a* and *b*.—An anaphase figure with 16 chromosomes at each pole.

FIGURE 20, *a* and *b*.—15 chromosomes are shown at each pole of this anaphase figure.


13
14a
14b
15
16
17
18a
18b
19a
19b
20a
20b

EXPLANATION OF PLATE 5

FIGURES 21 and 22.—Side views of the spindle of the heterotypic mitosis showing a chromosome passing undivided to one pole. The split shown in this odd chromosome is the line along which division will occur in the homotypic division.

FIGURE 23.—A drawing of the chromosomes at interkinesis following the heterotypic division. This figure is a representative of the 7-chromosome class of nuclei following reduction. Each chromosome is split forming the typical (for Oenothera) X-like structure. These splits are the same as the one described under figure 21 and 22.

FIGURE 24.—Side view of a spindle of the heterotypic division showing one chromosome which has failed to pass to either pole of the spindle.

FIGURE 25.—This shows an example of the 8-chromosome class of nuclei following reduction.

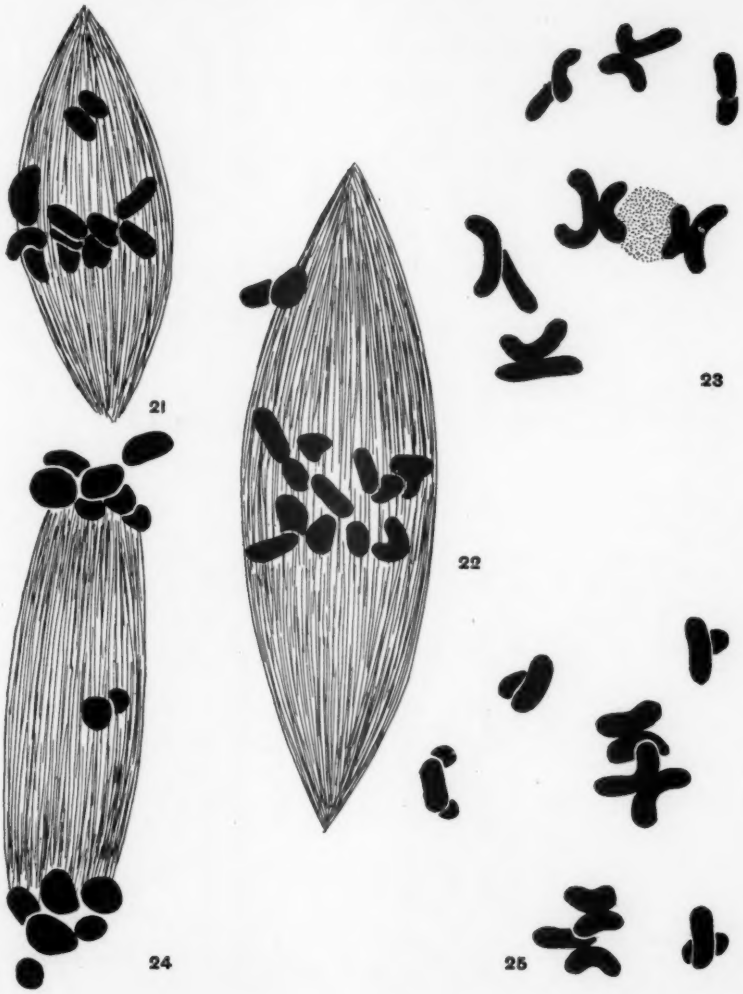

21
22
23
24
25

TABLE 13

The length of the chromosomes on plate 6.

The measurements were made on drawings of the chromosomes enlarged to 17,000 diameters.

Row number	Number of chromosomes	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	15	7	7	6.5	6.5	6.3	6	6	5.5	5	5	5	4.9	4.8	4.5	4.5						
2	15	7	6.7	6.5	6	6	5	5	5	4.8	4.8	4.8	4.5	4.4	4.3	3.5						
3	15	6.8	6.3	6	6	5.8	5.2	5	5	5	5	5	4.5	4.2	4.2	3.5						
4	15	7	6.1	5.6	5.6	5.2	5.1	4.8	4.3	4	4	3.8	3.8	3.8	3.2	3						
5	15	5.8	5.7	5.4	5.2	5.1	5	4.6	4.6	4.6	4.5	4.5	4.3	4	3.3	2.5						
6	15	6	5.8	5.8	5.6	5.6	5.3	5.1	5	5	5	4.8	4.5	4.5	4	3.8						
Average Length		6.6	6.3	6	5.8	5.7	5.3	5.1	4.9	4.7	4.7	4.7	4.4	4.3	3.8	3.5						
Maximum Length		7	7	6.5	6.5	6.3	6	6	5.5	5	5	5	4.9	4.8	4.5	4.5						
Minimum Length		5.8	5.7	5.4	5.2	5.1	5	4.8	4.3	4	4	3.8	3.8	3.8	3.2	2.5						
7	16	7	6	6	5.8	5.5	5.5	5.2	5	5	4.6	4.6	4.5	3.8	3.5	3.2	2.5					
8	16	7	6	6	6	6	5.6	5	4.8	4.8	4.8	4.8	4.5	4	3.8	3.6	2.4					
9	16	5.6	5.5	5.3	5.3	5	5	4.8	4.7	4.7	4.6	4.5	4.5	4.5	4	3.5	3					
10	16	6	6	5.5	5.3	5.2	5.2	5.1	5	4.8	4.8	4.6	4.5	4.5	4.2	4	3					
11	16	6.3	6	5.1	5.1	5.1	5	4.6	4.4	4.1	4	4	4	4	3.8	3	2.6					
12	17	6.5	6	6	5.8	5.3	5	5	5	4.3	4.3	4.2	4	4	3.8	3	3	2.2				

A B C D E F G H I J K L M N O P Q R S T U

TABLE 14

The length of the chromosomes on plate 7.

The measurements were made on drawings of the chromosomes enlarged to 17,000 diameters.

Row number	Number of chromosomes	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
13	17	4.5	4.1	4.1	4	4	3.8	3.6	3.5	3.5	3.3	3.3	3.2	3.1	3	3	3	2.5				
14	17	6	5.2	5	5	5	4.8	4.5	4.5	4.5	4.5	4	3.8	3	3	3	2.8	2.4				
15	17	7	6.9	6.8	6.6	6.3	6	5	5	5	5	4.8	4.6	4.5	4.4	4	3	2.8				
16	17	7	6.3	6	5.8	5.5	5.3	5.1	4.6	4.6	4.6	4.5	4	3.8	3.5	3.3	3.1	1.7				
17	18	6	6	5.5	5.2	5.2	4.8	4.8	4.5	4.3	3.5	3.4	3.1	3	3	2.5	2.5	2	1.7			
18	18	5.3	5.1	5.1	5	4.8	4.8	4.8	4.6	4.5	4.5	4.5	4	3.2	3.1	3.1	3	3	2			
19	18	5.8	5.1	5	5	4.8	4.5	4	4	3.8	3.6	3.6	3.5	3.3	3	3	2.8	2.5	2.2			
20	19	7	5.8	5.8	4.5	4.1	4	4	4	3.8	3.8	3.8	3.6	3.3	3.2	2.8	2.3	2.1	2	1		
21	19	5	4.6	4.5	4.3	4.3	4.2	4	4	3.8	3.5	3.3	3	3	2.8	2.5	2.5	1.8	1.5	1.5		
22	19	6	4.8	4.5	4.3	4.2	4.2	4	3.8	3.8	3.8	3	3	2.8	2.4	2.2	2	1.8	1.8	1.5		
23	20	5.5	5	5	4.8	4.8	4.5	4.2	4.2	4	4	3.8	3.8	3.2	2.8	2.5	2.3	2.3	2	1.8	1.5	
24	21	6	5.5	4.8	4.1	4	4	4	4	3.8	3.7	3.7	3.2	3	3	3	2.9	2.1	2.1	2.1	2	1.4

A B C D E F G H I J K L M N O P Q R S T U
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FEMINIZED MALE BIRDS

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[Received July 20, 1917]

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INTRODUCTION

The implantation, attachment and growth of ovaries in the body of the male has been successfully accomplished by several workers. MEISENHEIMER (1909) and KOPEC (1910) castrated young male caterpillars and implanted ovaries. The ovaries continued to grow in the foster soma. but no influence on the secondary sexual characters was observed. STOCKARD (1911) has reported successful results in implanting ovaries on the testis of the male in Diemyctylus, but as he makes no statement in regard to any effect on the sexual characters, it may be inferred that none were observed. Although the stroma of the ovary survived for several months the germ cells degenerated sooner. STEINACH (1912, 1913, 1916), working with rats and guinea pigs, secured the successful im-

plantation of ovaries into castrated males. In successful instances, the animals became completely feminized, not only in respect to morphological secondary sexual characters, but also in respect to behavior and the secretion of milk. STEINACH reports, however, that the germ cells always degenerated leaving only the interstitial gland which became hypertrophied. GOODALE (1916 a) has reported an instance of the successful engrafting of the homologous ovary into a castrated male fowl. The plumage, together with comb and wattles of the bird, was completely feminized, but the size, development of spurs and behavior each remained like that of the cock. In this individual, the ova developed to a size easily recognizable with the naked eye.

Before proceeding to a description of the various instances of feminization recorded in this paper, it may be well to recall the manner in which the characters of the capon differ from those of each of the two normal sexes, and to describe the appearance of the two types of feminized cockerels observed.

When the testes are completely removed from a young male fowl, the castrated individual at maturity is very much like a cock bird externally except in the following points. First, his comb and wattles, though present, are undeveloped; second, his plumage is longer than that of the cock, so that an *intensification* of *male* characters has resulted from the removal of the male primary sexual organs. The explanation of the long feathers, however, is probably merely another phase of the well known increased size of the capon over the cock, due to a continued growth of certain bones and associated parts, and therefore is to be looked upon as the result of the continued action of certain juvenile qualities. These long feathers, however, always develop after each molt, even in capons 3 or 4 years old. There is also a difference in the growth of primary coverts, which become twice as long in the capon as in the cock. The capon may exhibit all phases of the behavior of the cock, although ordinarily they are not manifested.

The capon (plate 1, fig. B) is entirely unlike the hen in appearance and, so far as I am aware, the only female character (except perhaps increased fat deposition) that he exhibits is his willingness to accept a flock of chicks and his behavior in caring for them. But as the normal males of some species of birds perform the same sort of function, the femaleness of this behavior may well be open to question.

In caponization it not infrequently happens, even with the best of care, that one or more minute pieces of the testes are not removed. These pieces may remain in a passive condition for months, and the birds

externally will have every appearance of being good capons. After varied lengths of time, however, the tissue may become active and then signs of its activity become visible externally, most noticeable in a partial or complete development of the comb and wattles.

The feminized cockerels are entirely unlike the capons in appearance, although the two have some characters in common. Their frames have always reached proportions similar to those of the male. Their weight, 4½-5½ pounds against 2½-4 pounds for normal females, corresponds to that of the normal male. They possess well developed spurs which are like those of the capon or cock. Their behavior, too, is very similar to that of a normal male. Their comb and wattles, however, have always approximated those of the hen in size and, in the single-combed feminized cockerels, have a lop very similar to that of a normal single-combed female. The plumage, likewise, has been entirely like that of the corresponding female except in two, perhaps three, instances, described below as type 2. The feathers have the same shape, the same color, the same development of barbules, the same arrangement of colors as those of the corresponding female. They are, however, somewhat larger than those of a normal Brown Leghorn female, but the greater size seems clearly connected with the larger body size of these birds.

In the feminized cockerels called type 2, the plumage is somewhat variable in character. Its shape is the same as that of a normal female but is somewhat longer in those regions which bear long feathers in the normal male. Golden or red edging (much reduced in amount) appears on many of the dorsal feathers. One of these individuals is black throughout except for the narrow red edging. In another the plumage color is almost black, also with a similar red edging. The reason for this type of feminized male can only be surmized at present. Several possible explanations are offered below.

OVARIES GRAFTED INTO CASTRATED MALE CHICKENS

The 1915 series

In the late autumn of 1915 a series of experiments was made in removing both testes and then implanting a piece of ovary. This series yielded three nicely feminized birds, one type 2 bird (No. 3379), while a fifth died at ten weeks of age.

Of the three feminized birds, two have been autopsied,[1] while the third, as well as the type 2 bird, is still alive. Their histories follow:

[1] The dictionaries examined afford no sanction for the use of autopsy as a verb. However, as this use of the word is convenient, the writer proposes that it be adopted.

No. 3897 was castrated October 19 at 30 days of age and two pieces of the ovary of a litter sister implanted on the right side only, together with the oviduct. The bird died February 21, apparently from exposure. The bird was clearly feminized externally, although it had not yet attained the adult plumage. On making the autopsy a piece of ovary was found attached to the surface of the right adrenal, and its ovarian character confirmed by a study of sections. The left side was empty. Search was made for the implanted oviduct but none found.

No. 3696. This individual was castrated October 20 as a young chick (31 days of age) and the ovary from a litter sister cut in very small pieces was implanted on the left side only. The plumage, both in respect to color and shape, and the head furnishings of this individual were completely feminized. The spurs were, however, well developed, while the bird had the large frame of a normal male. No signs of sexual activity were ever noticed. August 1 the bird was found dead on the roost in the morning and was at once autopsied. Death was due to suffocation from diphtheritic roup. The bird was very fat, quite like a hen. The right side was empty, but on the left, attached to the suprarenal and the adjacent parts of the vena cava and the transverse septum was a large mass of ovary very nearly of the same size as that of a normal female but separated from the body cavity by a fold of peritoneum. It is important to note that, because of the position of the ovary, no oviduct was found in this individual. The blood supply of the implanted ovary appeared good, as there was a fair-sized blood vessel leading from it to the vena cava. This implies a good supply of arterial blood, but the size of the artery or arteries supplying the ovary was not ascertained. The entire ovary appeared well nourished. There was a large number of good-sized ova, the largest of which reached 5 mm in diameter, while one abnormal ovum, such as are occasionally encountered in normal females and which appeared to be undergoing resorption, measured 10 mm in largest diameter. No empty follicles, however, could be found nor were there any other evidences that the ovary had produced full-sized ova and discharged them into the body cavity. Besides the ova described, there were a number of cysts filled with a serous fluid, the largest being 20 mm in diameter.

No. 2865. This bird, shown in figure E, plate 1, is the only one of this set remaining alive. Externally the description of 3696 applies to 2865. The method of castrating and implanting the pieces of ovary was the same except that the pieces were implanted on the right side instead of the left. At present, however, the conditions within the bird are

unknown. No signs of sexual activity have ever been noted in this individual.

No. 3473. Externally this bird, though quite young (10 weeks) at the time of its death, was entirely like the other young males and capons in the same flock. The sites of the testes were bare except for a bit of tissue on the left, a portion of which proved on sectioning to contain ova. There was no oviduct. It was impossible, however, to make out whether or not this tissue was in process of degeneration or was merely maintaining itself.

The type 2 bird of this set, No. 3379, was castrated October 22, when between 61 and 75 days of age and a part of the ovary of a litter sister implanted in the abdominal cavity of each side and also in the lung and kidney. A photograph of the bird is shown in figure F, plate 1. The bird has the general appearance of a hen except in the length of some of its tail feathers and the hackle feathers. Although a Brown Leghorn, it is black both dorsally and ventrally, with just a trace of golden red edging to some of the rump feathers. The comb and wattles are smaller than those of many rose comb Brown Leghorn females, but not smaller than some individuals. They are larger than those of a capon.

The 1916 series

The results of the experiments of 1916 are shown in table 1 from which, however, have been omitted several records of birds which died before reaching an age sufficient to determine whether or not male or female characters were to appear. Of the fourteen trials recorded in the table, six were successful; four were feminized to the type shown in figure D, plate 1, while the other two have been classified as type 2.

In this set the chicks were all pedigreed, that is, the father and mother of each is known. It is rather noteworthy that of the six successful instances, the ovaries used in four came from two daughters of one hen, the ovary from each daughter being divided between two males. This suggests that the ovaries of some individuals have a greater capacity for survival in the male than others. It is of further interest to note that both type 2 birds in this lot contained the ovary from one of these daughters while the two males containing ovary from the other daughter of 294 were of the more completely feminized type. Two of the feminized males were full brothers. All but one of this set have reached sexual maturity at this writing and this one (plate 1, fig. C), although externally possessing all the characters of a young pullet until well into the fall is now reverting. That is, as the adult plumage comes in, the feathers are

TABLE I

1916 series of experiments in feminizing cockerels.

Band No.	Mother's number	Date of operation	Nature of removal of testes	No. of female from which the ovary was taken	Mother's number	Results	Remarks
8442	5911	May 11	Both	8449	5824	Capon	
8444	277	" "	"	8456	5919	"	
8469	5920	" "	"	8456	5919	Feminized, type 1	
8470	5920	" "	"	8467	5925	Capon	
8471	5920	" "	"	8459	C 87	"	
C141[2]	. . .	" 24	Both—epididymi left	8872	294	Feminized, type 1	Reverted to capon, fig. C, plate 1
8866	5925	" "	" "	8863	C 84	Capon	
8868	291	" "	Both	8873	294	Feminized, type 2	Figure A, plate 1
8869	291	" "	"	8867	291	Capon	
8871	287	" 26	Both—no recorded statement about epididymi	8861	5915	Feminized, type 1	
8874	294	" "	Both—epididymi left	8861 8860 8862	5915 C 84 5915	Capon	Oviduct of 8861 implanted
8877	287	" 24	Both	8872	294	Feminized, type 1	Figure D, plate 1
8885	C123	" "	"	8873	294	Feminized, type 2	
8888	5920	" "	"	8867	291	Capon	

completely male-like. Doubtless, the implanted ovary has died, after living for several months in its foster soma. The plumage of the other individuals of this series in general agrees with the description given for the older set, save that four are not typical Brown Leghorns but resemble the extracted types of females noted below.

These four are all large-framed birds, weigh about the same as cockerels of this breed of the same age and their spurs are developing well.

[2] C 141. This individual was not pedigreed. Nor was it banded till nearing maturity. Its identification was established by its being the only unbanded chick.

Three are single-combed and it is interesting to note that these combs in size and shape and lop are wholly feminine in character. The behavior of this set of birds is very extraordinary, in that they constantly tread the hens. The act is not performed in the reflex manner that capons exhibit (GOODALE 1916 c), but in very nearly the same fashion manifested by the normal cock. The feminized cockerels sometimes crow but ordinarily they do not, nor do they exhibit much of the by-play of the normal cock, but they approach the females with every evidence of desire and go through all phases of copulation. In this respect they differ from the feminized males of the 1915 series (which, as far as observed, never trod the hens), but resemble the first cockerel feminized. An explanation of these differences between the various sets will not be attempted till more data are at hand. Attention, however, must be directed to the circumstance that the presence of ovarian tissue in the body of a male should accentuate the sexual behavior of the male, for while the male soma (i.e., a capon) in the absence of either gonad, may give the same reaction as an intact bird, as a rule there are few manifestations of it. In giving this brief discussion of the behavior of feminized males, it should be clearly understood that their behavior has all the characteristics of a normal male, save in some very minor details, but that it is given expression much less freely.

The existence of two sorts of feminized males requires some comment. While they may, of course, represent only the extremes of a variable series, it is also possible that they are the result either of some peculiarity of the genetic constitution of the birds or else due to the manner in which the operation was carried out. Of several possible explanations, the first to be considered is that only a portion of the cells of the ovary survived the implantation, and that they are not sufficient to entirely control the sex characters. Another possible explanation is that there is a different genetic basis in each of the two principal types and that each reacts differently to the same secretion. A third possible explanation could be offered, provided a portion either of the testes, epididymis, or both, were not removed, so that the birds are artificial hermaphrodites. Of the three possibilities the second seems the most probable at this writing. The stock from which these birds were reared contained, in addition to pure Brown Leghorns, several Brown Leghorn females extracted from a cross of this breed with White Plymouth Rocks. Now, many strains of Brown Leghorns produce an occasional female which has a brown instead of salmon-colored breast. While the extracted Brown Leghorn females

may have typical plumage, in some individuals the stippling is very much reduced, while in others, the salmon breast is dull-colored. Further a single female (a litter sister to some of the feminized cockerels) appeared in which the reduction of stippling was carried to an extreme degree. This bird has a dull black breast and is black dorsally, except for a very small amount of stippling along the margin of the feathers. This type of female plumage while not identical with the type 2 feminized male plumage is so close to it as to make it probable that the two are genetically the same.[3]

IMPLANTATION OF OVARY IN THE PRESENCE OF THE TESTIS

Since it has been demonstrated that ovarian tissue is capable of growth in the body of the male bird after the testes have been removed, it seemed desirable to attempt to secure ovarian grafts in the male without removing both testes. A number of experiments with this end in view were carried out in essentially the same fashion as for complete caponization except that only one testis (the right) was removed. Sometimes as much as possible of the epididymis was removed, sometimes no attempt was made to remove it. The ovary was then implanted on the site of the testis that had been removed or in the immediate neighborhood. The experiment was performed on eight birds, while a modification of the experiment was tried on two others. In one of the latter, neither testis was disturbed but the ovary was dropped into the body cavity, while in the other, the right testis was injured by pinching it with the forceps before implanting the ovary. This set of experiments is shown in table 2.

All ten birds were kept till mature and all developed outwardly into normal, vigorous males. The results are quite in contrast with those obtained when the males were first completely castrated. All but one individual have been subjected to autopsy. In 7 no trace of ovarian tissue could be found. In two instances (one doubtful, as the material has not yet been sectioned), ovarian tissue was found. This individual, No. 7950, externally was exactly like the other members of the series However, at the autopsy, a bit of tissue was found on the right side, that on sectioning proved to contain ova. While the ova could be distinguished with the naked eye, they were so much imbedded in other tissue that they could not be identified positively without sections. The whole mass appeared poorly nourished, as the vascular system was not visible to the naked eye.

[3] During 1917 a new type of plumage, nearly black in color, has been extracted from this strain of Brown Leghorns.

TABLE 2

Implantation of ovary in the presence of the testis.

A. Hemi-castrated males.

Band No.	Date of operation	Nature of the removal of the testis	No. of female from which ovary was taken	External appearance	Remarks
	1916				
7950	May 9	Right; epididymis left	7947	Normal male	Ovary found (see text)
7956	" "	Right; epididymis left	7973	" "	
7961	" 5	Right	7986	" "	
7965	" 9	Right and epididymis	7973	" "	
7976	" 5	Right injured by pincers but not removed	7981	" "	
7978	" "	Right, except for a small piece left free in the body cavity	7948	" "	
7979	" 9	Right and epididymis	7947	" "	
7980	" "	Right; epididymis left	7982	" "	
7987	" "	Right; epididymis left	7982	" "	

B. Male not castrated

7968	May 9	Both testes left	7948	Normal male	

The testis of this individual was about half the size of that of a normal cock of the same age. The vas deferens was not well filled, but a drop of the seminal fluid contained some motile spermatozoa.

Owing to the condition of the ovary, it seems hardly worth while to discuss the relation between the simultaneous presence of both male and

female gonads in a single individual and the secondary sexual characters. An ovary, externally like the piece found in this individual, has been found in feminized cockerels, along side of ovary that is normal in appearance. The possibility of producing artificial avian hermaphrodites, however, is clear.

FEMINIZED DRAKES

Feminization experiments have been carried out on five non-pedigreed Gray Call drakes. The Gray Calls (plate 2, G, male; H, female, which are colored like the Rouens, are practically domesticated Mallards.[4] In making the ovarian grafts, both testes and as much of the epididymis as possible were first removed and then the ovaries, cut in small pieces, from two litter sisters (in one case from only one female) were placed (either loosely or in pockets) in various positions in the anterior parts of the abdominal cavity. Of the five birds, three are definitely feminized. One shows no sign of being feminized and one is missing. The descriptions of the external appearances of the feminized birds (plate 2, figures I and J), are as follows:

No. 2890. The head and neck, i.e., that portion of the normal male which is bright green, is colored like that of the normal female. There is no white neck ring and the curl in the tail feathers is absent. The rest of the plumage is difficult to describe simply, as it is very complex but may, perhaps, be described by stating that superficially it is somewhat like the breeding plumage of the normal male, but much toned down by the addition of brown or dusky colors. The general color effect, however, is more like the color of a normal female. The individual feathers of the breast are claret or reddish, but have black markings, mostly splashes. The ventral feathers are vermiculated but they have either brown or black markings, or else they are washed with brownish or fuscous tones, according to the particular portion of the body to which they belong. Some individual feathers however, are strikingly like those of a normal female while a few are entirely masculine in character. The voice of this bird is not the *hiss* or "qua" of the normal male, neither does it possess the full, resonant qualities of the quack of the normal female, but occupies an intermediate position, perhaps best described as a broken quack. The upper mandible is colored exactly like that of a normal male.

No. 2877. Head female in color. There is no neck ring, while the curl is absent from the tail feathers. The remainder of the plumage in general is like that of the preceding individual but differs somewhat in detail. The

[4] For a description of the Gray Calls, see the American Standard of Perfection.

TABLE 3

Experiments in feminizing drakes (1916).

Band No.	Date of operation	Nature of removal of testes	Female from which ovary was obtained	Results
2877	July 27	Both testes and epididymi	3253	Head female, rest modified male
2890	" "	" "	2980	Head female, rest modified male; broken quack
2938	" 25	Both; no record for epididymi	3212 2942	Bird missing
3058	" 27	Both testes and epididymi	3657	Male characters only
3122	" 22	Both and part of the epididymi	2814 3816	Head female, rest male except new feathers; partial quack

voice of this bird is not altered from that of the male. The upper mandible is purely male in color.

No. 3122. The description given for 2890 applies equally well to this individual.

At this writing the birds are about one year of age, so that further changes in their plumage may be expected. In view of the possibilities of further change, only a few of the more peculiar points will be discussed at this time. In the first place, it is noteworthy that the head plumage of the birds should be more completely feminized than the remainder of the bird. It is true that the head of the young male is striped in a fashion similar to that of the female, but the individual feathers of the young male's head are usually colored somewhat differently, so that one familiar with these birds can pick out the sexes as soon as the birds are out of the down. In the feminized males, the head certainly has much more the appearance of a female than that of a young male.

In the absence of the neck ring and the curl of several of the tail feathers, the birds are female-like. The feathers which normally curl up are present and not only are not curled but in addition are brown and buff with the colors arranged in spots or splashes, instead of being uniformly black as in the normal male.

The pronounced masculine character of the color of the upper mandible

is in striking contrast to the feminization of the head plumage. The young ducks when first hatched all have black mandibles which in the male gradually become transformed into a light green with a small central black blotch. (This blotch is entriely absent in male Rouens). In the female, the black color persists, except for the extreme tip and margin of the mandible which are dark green. Although these ducks were quite young when operated on, no record of their exact age or of the color of the mandible was kept, so that it is possible that the color changes, which perhaps are irreversible, had already taken place. In castrated individuals of each sex, no change in the mandible color occurs except that a certain greenish cast in the female's mandible color disappears, leaving the mandible black and yellow. It seems possible that the color of the mandible may not be affected by the secretions of the ovary.

The imperfect quack emitted by two of the feminized males is of a good deal of interest, since the castrated individuals of each sex have always maintained their own voice.

GENERAL DISCUSSION

Regulatory effect of the gonadial secretions on growth

The studies made by various authors on ablation of the gonads in birds, combined with hetero-sexual transplantation, show that both the testes and ovary in birds produce substances that have a profound regulatory effect on growth. Of the two organs, the ovary produces the substances of most interest, for except in the case of the hen-feathered males described by MORGAN (1915) the secretions of the testes affect the *extent* of growth, either by inhibiting growth at a definite point, such as feather length, bone length, or else by furnishing substances necessary for the continued growth of certain organs, such as comb and wattles. The ovary, on the other hand, not only furnishes substances that determine the *amount* of growth, but also substances that determine *what sort* of growth takes place within the limits furnished by the genetic constitution of the bird. The substances produced, moreover, seem to have a specific relation to various organs, which is discussed below.

Differences between feminized birds and mammals

The results secured with birds differ in some important respects from those secured by STEINACH for mammals. According to STEINACH (1916), the rats, guinea pigs and rabbits used by him, were completely feminized, not only in respect to body conformation but also in respect to

behavior. In the birds, however, the feminization has been confined to certain organs or sets of organs, while others have not been feminized. In behavior, moreover, the birds have either been neutral or have exhibited the sexual behavior of the male.

Each character a unit

In so far as the individual characters can be picked out, each is either feminized or not feminized.[5] In other words, the bird behaves as though composed of separate units in much the same fashion as Mendelism has shown us regarding the inheritance of ordinary characters. The various characters, however, fall into several groups. We may recognize, first, characters (including some of the secondary sexual characters) that are independent of either ovary or testis. Such characters are: size in the female, voice and some phases of behavior, and mandible color in ducks; second, characters affected by the testes, such as comb and wattles, fat deposition, size in the male, some instincts and summer plumage in ducks; third, characters that are affected by the ovary, such as plumage form and color, and some phases of behavior.

No sharp line can be drawn between the better known secondary sexual characters and those commonly considered ordinary somatic characters, for the reaction of a character to the secretion of either gonad varies not only according to the character itself, but also according to the original sex of the individual. Thus, the size of the primary coverts in relation to the primaries is approximately the same in each sex when intact, but after the removal of the testes they become disproportionately large, though not after the removal of the ovary. Thus castration of the male, curiously enough, produces an unique secondary (sexual?) difference, unique because it has been produced artificially. The spurs always develop in the female after the removal of the ovary, but they also develop in the capon and in feminized cockerels, i.e., in the presence of the ovary in the soma of the male. Females from which the ovary has been removed are neutral in sexual behavior, but one of the most astonishing things about castrated males with implanted ovaries is that they exhibit the sexual behavior of the male only. The comb and wattles do not develop in the male after castration, i.e., are infantile; in the castrated

[5]It is a matter of some possible importance to note that races in which the males lack horns or spurs, as the case may be, while the female possesses such structures, seem to be unknown, i.e., one of the four possible combinations, viz., spurred males, non-spurred females; both sexes spurred; both non-spurred; males not spurred, females spurred; is not realized.

male with engrafted ovary they are fully feminized; in the ovariotomized female they may be either female-like or male-like. The capon exhibits two characters that are female-like, viz., the amount of fat deposited and the brooding instinct. On the other hand, two masculine characters are intensified by the removal of the testes, viz., body size and plumage length. The ovariotomized female is approximately the same size, however, as her normal sisters, while the castrated male with engrafted ovary has the size of the male. The plumage shape seems to be completely controlled by the ovary, since wherever that is present the shape of the feather is like those found on intact females. The color may be completely controlled by the ovary for while females from which the ovary has been removed develop the colors of the male, only a portion, type 1 feminized males, (the ducks and type 2, feminized cockerels forming the exception) of those males with engrafted ovaries have developed the female's color. In ducks there is a greenish pigment of the mandible that disappears from the mandible of the castrated female, but, since the mandible color of the male with engrafted ovaries is not feminized, it appears that castration of the female induces a previously non-existent difference between the sexes. Finally, there are characters that behave one way in some individuals and in another way in others. This is particularly noticeable in the plumage of the ovariotomized ducks, which in particular regions of some individuals, may vary from a purely masculine condition to a purely female condition or to a condition *sui generis*.

Size and the gonadial secretions

The body size of the male bears a definite relation to the secretions of the testis as shown by the smaller size of the intact male over the castrated male. On the other hand, the ovary has no influence on body size since castrated females have the body size of the female, while the feminized cockerels have the body size of the male. The remarkable results secured by PEARL (1916b), who has succeeded in eliminating the size difference between the sexes by feeding calcium lactate and calcium phospho-lactate, show clearly that size is a secondary sexual character of a different sort from the plumage. It may be assumed that the size differences are due to a gene that is linked with the "female" gene, so closely that they always go together, and that the calcium salts used altered the action of this gene just as other environmental factors may affect the expression of a Mendelian character. In this connection, it is of interest to note that the sex that has the smaller body size should

have the larger germ cells. The greater metabolic activity of the female in producing large amounts of stored food in the eggs is not ordinarily expressed in body size even when there is only a small amount of active metabolism in producing germ cells such as occurs during the growth of the young bird.

Head furnishings and the gonadial secretions

The relation of the head furnishings (comb and wattles) to the gonads now seems pretty clear. A basis for the comb is furnished by the hereditary make-up of the bird, but its character depends upon the particular gonad with which it is associated. If associated with the testis, a male comb develops; if with an ovary, a female comb develops. That is, the dimensions, texture, etc., and the lop of a fowl's comb are determined directly by the activity of the gonad with which they are associated. The fact that ovariotomized pullets often, but not always, develop large combs, is not contradictory to this statement because of the presence of the hypertrophied "Wolffian body" which affords the possibility at least of a substitute secretion for that of the entire gonad (GOODALE 1916 b). Another explanation (capable of definite proof or disproof) lies in the possibility that different kinds of combs react differently to the ovarian secretion. In some races of poultry the comb is small, in other races, large. Experience shows that small-combed individuals occur rather frequently in the large-combed races, and, as there is pretty definite evidence that some types, at least, of large combs differ from small ones genetically (unpublished experiments), it seems possible that the small-combed type may not be subject to the influence of the secretions of the ovary, while the large-combed type may be so subject. The former, therefore, after removal of the ovary, remains unaltered, while the latter, being freed from the influence of the ovarian secretion, develops to a large size. Of course, we are in entire ignorance regarding what particular cells of the gonad are responsible for comb structure.

Spurs and the gonadial secretions

The relation of the spurs to the ovarian secretion also is of peculiar interest, for, although the secretion is effective in suppressing the development of these organs in most normal females, it has not yet suppressed them in the feminized males. This behavior together with the occurrence of spurs in an occasional unaltered female, suggests that either the control exerted by the ovarian secretion is slight, or else that

certain germinal factors for spur development exist in different conditions (homozygous or heterozygous) in the two sexes or that additional germinal factors (inhibitors, for example) may be present, which react in a different manner to ovarian secretion. Castrated females which revert to female plumage almost always have very good spurs, which apparently continue to grow in spite of the regenerated ovary. This suggests that the former view is correct. On the other hand, the occurrence of spurs in the feminized cockerels and the existence of strains of domestic fowls in which the females are very likely to bear large spurs suggest the second alternative. The alternatives, however, are not mutually exclusive.

Behavior and the gonadial secretions

The behavior of the feminized males was described on page 282. It is essentially masculine throughout, but as in the capon, some phases of it rarely receive expression. The capon, however, exhibits a phase of behavior,—brooding of chicks,—ordinarily observed only in the female. The feminized males have not been tested for this point. The castrated female is essentially neutral in behavior, although the behavior on certain rare occasions has a pronounced masculine cast. However, even intact females occasionally exhibit such behavior.

It seems possible that the various phases of behavior may be separated from one another and that the observed behavior may depend in part on the presence of a definite genetic basis modified by an internal secretion. Thus the character of the sexual reactions seems to depend upon the substratum, while the gonad merely determines that it shall be given expression. The feminized cockerels crow so little that in practice they may almost be said to lack the crowing instinct. In this they resemble the capon. Here the necessary substratum is present, but the ovary does not furnish as suitable an activating agent as the testes. On the other hand, the feminized birds may "crrrrrrrk" (known to poultrymen as "singing"), a sound that normal males very rarely give expression to.

Color of the feminized males

As suggested, the exact color taken by the feminized cockerels may depend upon their genetic constitution. Thus, it was noted for the feminized cockerel first described (GOODALE, 1916) that "the feathers of the dorsal regions were black with relatively few minute brown spots instead of the uniform mixtures of minute dull black and dark brown spots characteristic of the Brown Leghorn female." The portion of the quo-

tation referring to the characteristics of the Brown Leghorn females did not give sufficient detail, for an occasional female may appear in which the stippling is less pronounced, and if given opportunity, by laxity in selection of the breeders, or, as has happened in the present series through the use of extracted recessives, may appear in considerable numbers. It is clear, however, that except for the type 2 feminized males, the plumage becomes entirely feminized.

Nature of the secretion of the gonads

It is rather difficult to believe that the secretion of either the testis or the ovary is a simple substance, because of the diverse effects produced.[6] Thus, the secretion of the testes serves both to limit growth, as shown by the shorter bones and plumage of the cock compared with the capon, and also to accelerate growth, as shown by its effect on the comb and wattles, which are large in the cock and undeveloped in the capon. The secretion of the ovary, too, acts in some ways to limit the development of male characters, such as the complete inhibition of the spurs and the limitation of feather length. At the same time the secretion of the ovary is necessary for the development of the barbules of the feathers of the dorsal regions, since the barbules do not develop along the entire length of the barb in the male or in the capon. Since spurs may develop in otherwise normal females, support is lent to the view that the secretion is complex and that the special element that controls spur development is absent or else has undergone some change in the spurred females. Another view of the action of the secretions is also possible and is not contradictory to the first view. It assumes that part of the apparent complexity of the secretions is not real but is due to differences in the substratum on which it acts. Thus, the presence of spurs in the otherwise normal female may be due to greater growth possibilities in the spurs of spurred races, and this view is supported by the failure of implanted ovaries to suppress spur development in the feminized males. But while both views may be applicable in the case of the spurs, the latter view appears to be inapplicable in the case of the barbules of the

[6] This statement is not really contradictory to a statement made in a previous paper (GOODALE 1916 c) to the effect that the effects produced by the ovarian secretion should be considered to be due to a simple substance. Clearly this is not the case at least as far as the large groups of characters are concerned, for while the ovarian secretion may be complex in the sense that it contains one substance for the control of the plumage, another for the head furnishings, and so on, each substance may react with a specific hereditary basis in producing the diverse conditions such as the various colors of the plumage observed in the various varieties.

saddle feathers which develop only when the secretion of the ovary is present, except in the case of hen-feathered males and here it seems clear from MORGAN'S work (MORGAN 1915) that the secretion that is found usually in the female occurs also in the hen-feathered male. On the whole it seems probable that the secretions of both testes and ovaries are not homogeneous but possess a certain amount of complexity.

Certain aspects of the sex question

There are two aspects of the sex question that should be kept distinct, viz., secondary sexual characters should not be confused with the primary sex characters. Thus the former are commonly found associated with one sex but not the other. To assume (as RIDDLE (1914, 1916) seems to have done) that such characters themselves acquire sexuality, i.e., are either male or female, seems entirely gratuitous. They may, indeed, be closely associated normally with a particular kind of gonad, but since other characters that are wholly independent of sex may be linked together, it is clear that this linkage affords a reasonable explanation of the association of certain characters with a particular sort of gonad, but when in addition we find certain of these characters, associated sometimes with one gonad and sometimes with another (feather form in hen-feathering for example), or in association with both gonads (horns for example), it becomes clear that such characters are not of value as an index of the masculinity or femininity of either themselves or the individual that bears them.

The second aspect of the question relates to the primary sex characters. Here a series of intergrades in the primary sex organs has been obtained by BANTA (1916) in Simocephalus and GOLDSCHMIDT (1917) in Lymantria, in addition to a series of intergrades among the secondary sexual characters. The intergradation of sex appears in a series of hermaphrodites, in which at one end the gonads produce nearly all of one sort of sex element, and at the other nearly all the product is of the other element. These, then, are true sex intergrades, not merely intergrades in secondary sexual characters.

From the descriptions that have been given it will be observed that the feminized males may be described as a series of secondary sex intergrades, or secondary sex mosaics, ranging from birds that are comparatively little feminized (type 2) to those that have exhibited very few male characters.

If the various characters are independent units from the standpoint of heredity, and if they depend in various degrees upon the secretions of

certain cells or groups of cells, these groups themselves perhaps being independent in heredity, i.e., each group forming a physiological unit, comparable to independent external morphological units, such as comb, feathers, etc., and if these are linked to the sex-determining chromosome in some manner, we can see how it is possible for various secondary sex intergrades to arise.[7]

There is another aspect of the question of the secondary sexual characters that should be pointed out. We know that certain characters, barring, spangling, etc., are inherited in a definite fashion, the female being heterozygous for the characters. These characters are dominant in heredity, but if they were recessive, then they might possibly appear as secondary sexual characters, since in a "pure" race only the males would be duplex and able to exhibit the characters in question, while the female, being simplex, would not exhibit it. Both elements, secretion and substratum, are approximately equally important in determining what sort of character actually develops.

SUMMARY

It is possible to remove the testes from a male fowl and secure long-time ovarian grafts.

In those instances that have been autopsied, no evidence was found to show that the ovary had ever discharged ova, although ova were found several millimeters in diameter.

The implanted ovary influences some of the secondary sexual characters, viz., comb, wattles, and plumage, causing them to become indistinguishable from the female form. Other secondary sexual characters, such as spurs, behavior, and size are either wholly unmodified, or, if modified, it is not in the direction of feminization.

Similar results were secured with ducks.

It was found possible to secure a persistent ovary in one instance in the presence of an active testis. The secondary sexual characters were unmodified, but the persistent testis was much below normal development, although producing motile spermatozoa.

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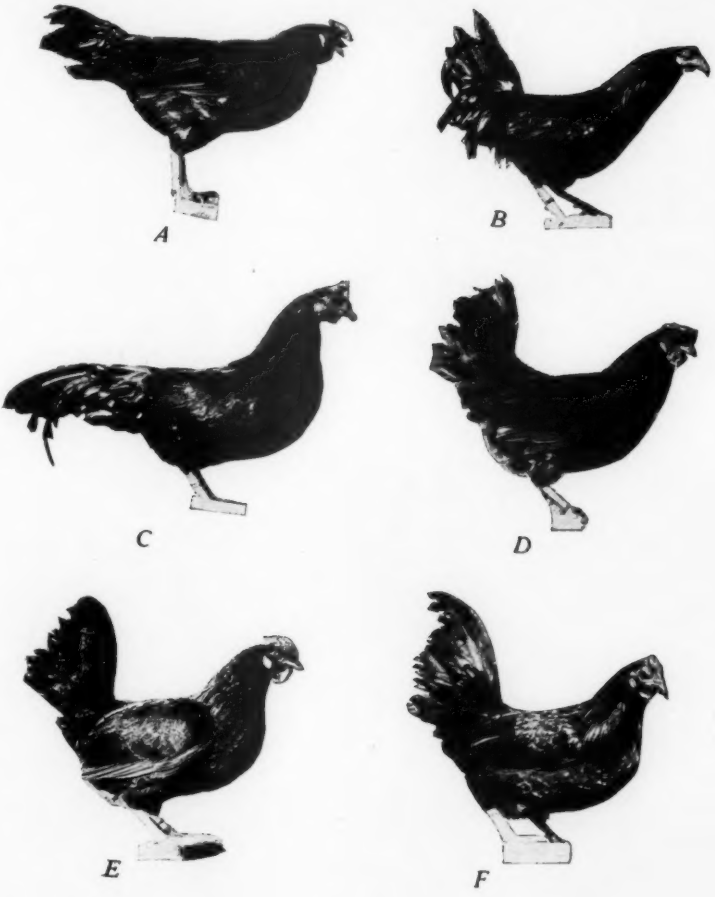
EXPLANATION OF PLATE I

A. Feminized cockerel No. 8868.
B. Capon.
C. No. C 141. This bird was fully feminized, but while changing to a capon's plumage, is passing through a mosaic condition, showing both male and female feathers intermingled.
D. Feminized cockerel No. 8877.
E. Feminized cockerel No. 2865.
F. Feminized cockerel No. 3379. Type 2.


A
B
C
D
E
F

EXPLANATION OF PLATE 2

G. Normal Gray Call drake.
H. Normal Gray Call duck.
I-J. Two feminized drakes. Note the female head plumage, the male mandible, and the mixed condition of the body plumage.

G

H

I

J

THE INHERITANCE OF DOUBLENESS IN *CHELIDONIUM MAJUS* LINN.

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[Received September 3, 1917]

The most thorough investigation of the inheritance of doubleness in flowers has been made by Miss SAUNDERS (1910, 1911, 1917). In the Petunia the double flowers set no seed and all crosses must be made with the single plant as the female parent. In the F_1 of such a cross there is either a segregation into 3 single : 1 double, or into 9 single : 7 double. All single plants selfed or crossed *inter se* produce only singles. SAUNDERS explains these results on the assumption that pollen from the single flower used carries only factors for singleness, while ovaries of the single flowers lack the factor for singleness in some cases and possibly in all. The pollen of double flowers is further assumed to be heterozygous. In the stock the inheritance of double and single flowers is explained on the assumption that two factors are involved which are linked in the pure singles, but not linked in the eversporting singles. It is also assumed that "single" factors are distributed only in the female gametes according to a system of partial linkage.

In the Welsh poppy (*Meconopsis cambrica*) SAUNDERS (1917) found that a simple 3 : 1 ratio was obtained in F_2 by crossing single and double plants. Doubleness was found to be dominant. In the hollyhock (*Althaea rosea, A. ficifolia*) the offspring of single $\times$ full double plants were intermediate in F_1 and in the F_2 produced a 1 : 2 : 1 ratio. In the carnation (*Dianthus caryophyllus*) a cross of double $\times$ single produced an F_2 ratio of 3 double : 1 single, while a cross of double $\times$ single in *Dianthus barbatus* (sweet william) gave the same F_2 ratio, but singleness was found to be dominant.

NORTON (1907) and BATCHELOR (1912) have found that doubleness in the carnation is of two types; the ordinary or standard double and the type known as "bullhead" or "buster". By crossing a full double or buster with the single the resulting F_1 is standard double. The F_1

selfed or crossed *inter se* produces an F_2 ratio of 1 single : 2 standard doubles : 1 full double or buster.

The doubling of flowers is usually due to petalody of the stamens, according to DE VRIES. This is true of the plants investigated by SAUNDERS, and has been recorded by many writers, GOEBEL (1913), MASTERS (1869), DE VRIES (1906), and others; but no statistical data have been presented to show the degree of correlation between stamen number and petal number.

The inheritance of doubleness in *Chelidonium majus* and a statistical study of the relation of petals and stamens will be considered in the present paper. A double- and a single-flowered plant growing near the BUSSEY INSTITUTION were transplanted and reciprocal crosses made. In the F_1 about sixty plants of each cross were grown and in each case produced practically an equal number of single and double plants. Seed from the F_1 singles and doubles were planted separately. Of 133 plants raised from seed of single F_1 plants, 109 were single and 24 double. Of 111 plants raised from seed of the double F_1 plants, 6 were single and 105 double. If we assume that the high number of singles resulting from F_1 singles, and the 6 singles among the doubles, were due to contamination by crossing, or possibly due to volunteers from wild plants growing near by, then it appears that singleness is dominant, and that the original cross was made with a heterozygous single. We would then expect a ratio of 1 : 1 in the F_1 irrespective of which plant is used as the female parent. In the F_2 the F_1 single segregates should give a ratio of 3 single : 1 double, while the F_1 double segregates should breed true, when selfed or crossed *inter se*. In the F_2 of this particular cross all of the doubles should be homozygous, while two-thirds of the singles should be heterozygous.

Of the 244 plants raised in the F_2 the petal number and stamen number of the flowers of 147 plants were found. An average of 20.6 flowers per plant were counted.

The average numbers of petals and stamens of the F_2 plants are shown in table 1. The mean petal number is 10.54 ± 0.31, with a standard deviation of 5.60 ± 0.22. The mean stamen number is 18.30 ± 0.26, with the standard deviation of 4.72 ± 0.19. The coefficient of correlation between stamen and petal number is $-.90 \pm .01$. In table 2 all of the flowers of the 147 F_2 plants are plotted in respect to stamen number and petal number. The mean petal number is 10.89 ± 0.07 with a standard deviation of 5.84 ± 0.05, while the mean stamen number is

TABLE 1

Average stamen and petal number of 147 F_2 plants. Chelidonium, single × double.

Petals \ Stamens	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
4											1	1	4	6	6	23	10	4	1	56
5																				0
6																				0
7																				0
8											1									1
9													1							1
10									2	1	1									4
11								1		4	1									6
12						3			2		1									6
13					2	3	1	2	5	2										15
14				4	2	2	4	7	1	2										22
15						3	1	1	1											6
16				2	2	1			1											6
17	1	1	1		2			2												7
18			2		2	1	2			1										8
19					3		1													4
20		1	1				2													4
21																				0
22						1														1
	1	2	4	6	13	14	11	13	12	10	5	1	5	6	6	23	10	4	1	147

Petals: M = 10.54 ± 0.31, σ = 5.60 ± 0.22

Stamens: M = 18.30 ± 0.26, σ = 4.72 ± 0.19

$r = -.90 \pm .01$

* Not weighted for deviation in number of flowers counted.

18.14 ± 0.06 with a standard deviation of 5.15 ± 0.04. The coefficient of correlation is — .863 ± .003. The variation of the doubles is especially striking in table 3, and there is apparently a continuous gradation from single to full double.

There is no significant variation of the petal number (four) in the singles, while the mean number of stamens is 23.68 ± 0.14 with a standard deviation of 1.56 ± 0.09. Although the doubles are recessive, the variation of petal number and of stamen number is much greater than in the singles. The F_2 doubles are plotted in table 3. The mean number of petals is 14.56 ± 0.20 with a standard deviation of 2.81 ± 0.14, and the mean stamen number is 14.99 ± 0.17 with a standard deviation of 2.39 ± 0.12. The coefficient of correlation is — .58 ± .05. It is apparent that the singles, even though two-thirds of them are heterozygous, are much less variable than the recessive doubles.

The sum of the petal number and stamen number is about the same in all individuals, whether single or double. The mean sum of the petals

TABLE 2

Stamen number and petal number of all F_2 flowers counted. Chelidonium, single × double.

Petals \ Stamens	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
3																		2	1								3
4											1		9	32	59	95	93	160	210	143	129	81	31	9	6	2	1060
5															2	2	8	6	2	2	2	3		2			29
6																1	4	1	3	1	1						11
7												1	1	1	2	1	8		2		2						18
8											1	1	3	2	7	8	4	1									27
9									2	1	2	3	6	4	6	1	6	1	1		1						34
10							1		4	6	8	6	10	7	6	2	1										51
11						1		4	4	9	19	15	16	15	4	4	2			1							94
12						2	13	18	32	38	50	40	49	26	17	14	3		1								303
13					8	3	17	11	38	26	43	34	27	15	15	7											244
14			1	2	7	2	28	11	46	35	41	20	20	8	5	3	1										230
15			1	1	9	13	23	26	29	22	31	14	16	7	1	2											195
16			4	7	11	9	24	27	27	24	25	10	5	5	5	3											186
17			4	1	11	13	13	17	16	9	23	3	3	2	2		1										118
18			7	5	9	13	22	15	21	6	7	5	8	1													119
19			7	8	16	16	23	6	15	4	4	5	1		1												106
20	3		8	9	9	9	17	8	7	6	6	7		1													90
21		1	2	3	3	8	10	8	6	4	4	1	1	1													52
22		3		2	2	3	4	3	5	2		3	1			1											29
23			1	1	2	2	2	1	3		1																13
24					1	1	1	1	1	2																	7
25						1	1	1	1																		4
26						1	2	2																			5
27									1																		1
	3	4	35	39	88	97	201	159	258	194	266	168	176	127	132	144	131	171	220	147	135	84	31	11	6	2	3092

Petals: $M = 10.89 \pm 0.07$, $\sigma = 5.84 \pm 0.05$

Stamens: $M = 18.14 \pm 0.06$, $\sigma = 5.15 \pm 0.04$

$r = -.863 \pm .003$

and stamens of the doubles is 29.49 ± 0.15 with a standard deviation of 2.24 ± 0.12, while in the singles the mean sum of petal number and stamen number is 27.68 ± 0.14 with a standard deviation of 1.56 ± 0.10. The sum of the petal number and stamen number in the doubles is significantly larger than in the singles, but with a much greater variation

TABLE 3

Average stamen number and petal number of F_2 double plants. From table 1

Petals \ Stamens	9	10	11	12	13	14	15	16	17	18	19	20	21	
8											1			1
9													1	1
10									2	1	1			4
11								1		4	1			6
12						3			2		1			6
13					2	3	1	2	5	2				15
14				4	2	2	4	7	1	2				22
15						3	1	1	1					6
16				2	2	1			1					6
17	1	1	1		2			2						7
18			2		2	1	2							8
19					3		1							4
20		1	1				2							4
21														0
22						1								1
	1	2	4	6	13	14	11	13	12	10	4	0	1	91

Petals: $M = 14.56 \pm 0.20$, $\sigma = 2.81 \pm 0.14$

Stamens: $M = 14.99 \pm 0.17$, $\sigma = 2.39 \pm 0.12$

$r = -.58 \pm .05$

If the pedigree culture results did not show the singles to be dominant, one might expect the larger number of petals and stamens and the greater variability of the doubles to be due to heterozygosis. The behavior of the doubles may, however, be due to splitting of the stamens in some cases, in addition to petalody.

The greater variation in number of parts in the double flowers loses its significance when the doubles of the F_2 are grouped by themselves and compared with the double flowers on individual double plants of the F_2. The mean petal number of all of the F_2 double plants is 14.56 ± 0.20 with a standard deviation of 2.81 ± 0.14 and the mean stamen number is 14.99 ± 0.17 with a standard deviation of 2.39 ± 0.12 (table 3). In tables 4, 5 and 6, three F_2 double-flowered plants are plotted in respect to petal number and stamen number. In table 4 the

TABLE 4

Stamen number and petal number of flowers of a single F_2 'double' plant.

Petals \ Stamens	10	11	12	13	14	15	16	17	18	19	20	21	
9									1				1
10													0
11													0
12					1	1						1	3
13				1	1			2			1	1	6
14					1	2	1	1		1			6
15								1	1	1		1	4
16					2	2	3				1	1	9
17				1	1	2	6	1		2			13
18			1	1	1			2	3	1			9
19		1	2		1	2	2	1					9
20			3	1	1	1	4	1					11
21	1		1	1	2	2	2						9
22				1									1
	1	1	7	6	11	12	18	9	5	5	2	4	81

Petals: $M = 17.23 \pm 0.20$, $\sigma = 2.77 \pm 0.14$

Stamens: $M = 15.64 \pm 0.18$, $\sigma = 2.43 \pm 0.13$

$r = -.46 \pm .04$

TABLE 5

Stamen number and petal number of flowers of an F_2 'double' plant.

Petals \ Stamens	8	9	10	11	12	13	14	15	16	
14					1	1				2
15						5	1		1	7
16			2	5	3	1	2	1		14
17			1	3	1			1		6
18	2		3	1	6			1		12
19	2	1								3
20	2	2	3		1					8
	6	3	9	9	12	7	3	3	1	53

Petals: $M = 17.15 \pm 0.16$, $\sigma = 1.71 \pm 0.11$

Stamens: $M = 11.36 \pm 0.18$, $\sigma = 1.98 \pm 0.13$

$r = -.64 \pm .05$

mean petal number is 17.23 ± 0.20 with a standard deviation of 2.77 ± 0.14 and the mean stamen number is 15.64 ± 0.18 with a standard deviation of 2.43 ± 0.13. In table 5 the mean petal number is 17.15 ± 0.16

TABLE 6

Stamen number and petal number of flowers of an F_2 'double' plant.

Petals \ Stamens	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
10									1		1				2
11							1	2		1	1			1	6
12								2		2	1	2	1		8
13							1	2	1	2	2				8
14						2	1	2	1	1	1	1			9
15							1	1		1					3
16			1	1											2
17															0
18					1										1
19	2														2
20															
	2	0	1	1	1	2	4	9	3	7	6	3	1	1	41

Petals: $M = 13.29 \pm 0.22$, $\sigma = 2.11 \pm 0.16$

Stamens: $M = 16.58 \pm 0.30$, $\sigma = 2.88 \pm 0.21$

$r = -.66 \pm .06$

with a standard deviation of 1.17 ± 0.11 and the mean stamen number is 11.36 ± 0.18 with a standard deviation of 1.98 ± 0.13. In table 6 the mean petal number is 13.29 ± 0.22 with a standard deviation of 2.11 ± 0.16 and the mean stamen number is 16.58 ± 0.30 with a standard deviation of 2.88 ± 0.21. The coefficient of correlation of all the F_2 doubles is $-.58 \pm .05$ while the coefficients of correlation of the three F_2 double plants are $-.46 \pm .04$, $-.64 \pm .05$, and $-.66 \pm .06$, respectively. The variability of the petal number and stamen number of all of the F_2 doubles is, in general, not greater than the variability in individual F_2 double plants.

CONCLUSIONS

Doubleness appears to be a simple recessive character in *Chelidonium majus*.

There is apparently a continuous series in degree of doubling from singles to full doubles in the F_2.

There is much greater variation in the doubles, which are recessive, than in the singles, of which two-thirds are heterozygous. The F_2 doubles are however no more variable than individual double plants of the F_2.

There is a high degree of negative correlation between petal number and stamen number in the F_2, due to petalody.

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